HANDBOOK OF PARASITOLOGY

A.K. Awasthi
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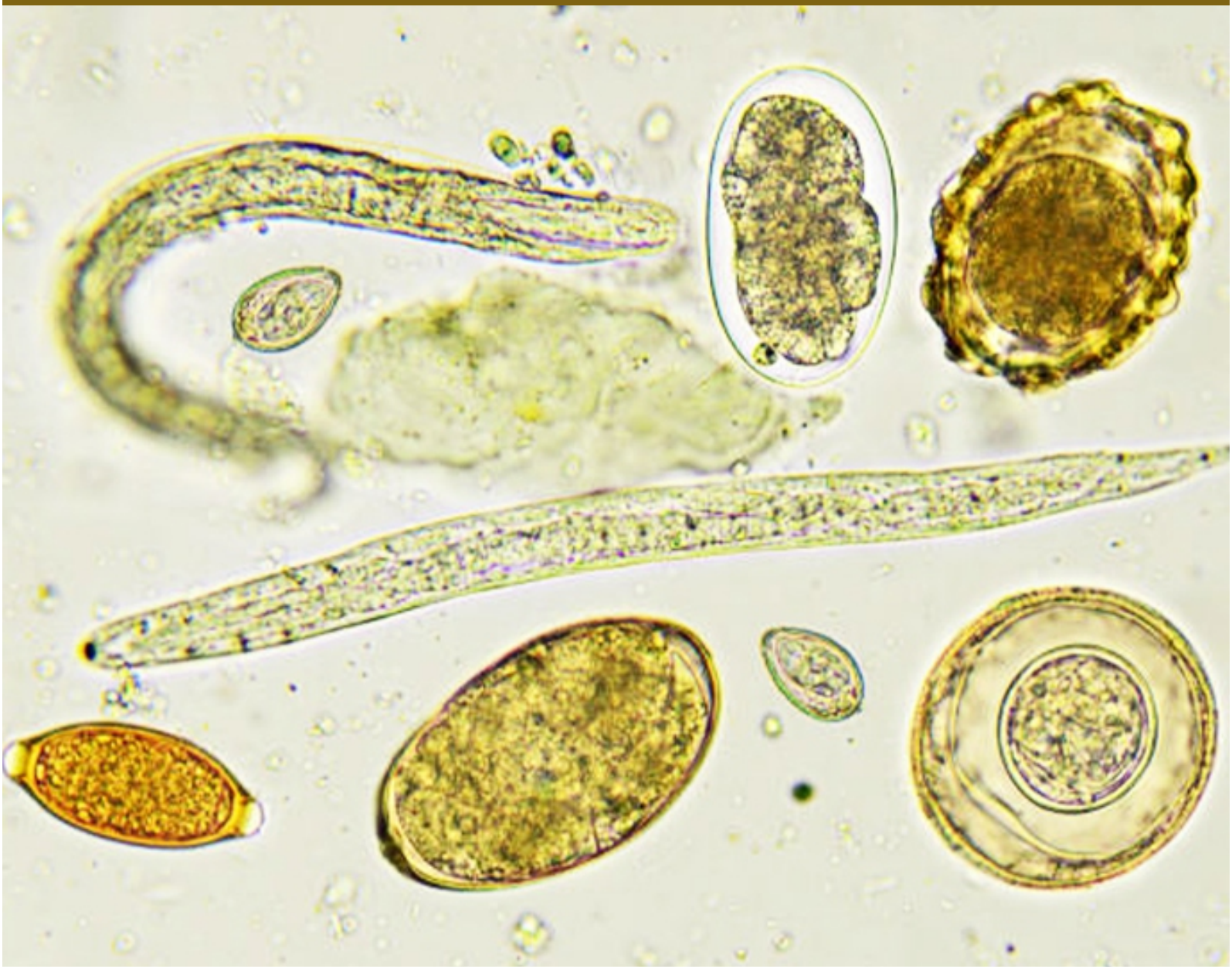

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CHAPTER 1

AN OLD AND NEW DEVELOPMENTS IN MALARIA'S LIFE CYCLE, PATHOGENESIS, DIAGNOSIS, PREVENTION AND TREATMENT WITH AN EMPHASIS ON ETHIOPIA

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ABSTRACT:

The apicomplexan parasite that causes malaria is an ancient illness that still poses a serious danger to the public's health in many nations. This page seeks to provide several elements of malaria in a clear and thorough way, including causes, pathophysiology, prevention, and therapy. There are six Plasmodium species that are known to cause human malaria, with Plasmodium falciparum being the most common in East and Southern Africa. The two most potent malaria vectors in the world, Anopheles gambiae and Anopheles funestus, are primarily responsible for transmitting malaria. Malaria infection poses a threat to half of the world's population. Though few surveys indicated a significant frequency of malaria in Ethiopia, the rates of morbidity and death from malaria have reduced globally. The malaria parasite has a convoluted life cycle that involves both mosquitoes and people. Malaria diagnoses may be divided into clinical and parasitological diagnostics in general. The endeavor to develop novel medications, vaccines, and malaria prevention strategies has been hampered by a lack of clarity on the general biology of Plasmodium. However, due to the emergence of resistance among widely used medications and insecticides, three different vaccine types and several new chemicals are now undergoing perclinical and clinical investigations. In the lab, antiadhesion adjunctive medicines are also being researched. Scientists from throughout the globe are looking for novel targets and chemical entities in addition to previously identified targets for diagnostic tools, vaccines, and drugs.

KEYWORDS:

Malaria, Medicines, Vaccines, Scientists.

INTRODUCTION

The word "malaria," which means "bad air," is Italian and is where the name "malaria" comes from. It is a protozoal blood infection brought on by an apicomplexan parasite that is carried by mosquitoes and spreads to people when they are bitten by infected female Anopheles mosquitoes. Malaria is a condition brought on by a parasite that spends some of its life in people and some in mosquitoes, according to the National Institute of Allergy and Infectious Diseases (NIAID) of the United States. This study seeks to provide a complete and cohesive overview of all aspects of malaria. An effort was made to provide basic information on the history, underlying causes, prevalence, and incidence of malaria. It also combines both outdated and contemporary ideas regarding malaria pathogenesis, diagnosis, and treatment under one roof, along with some advice from Ethiopia. We aim to outline new advances in malaria treatment, vaccination, and control methods in advance [1], [2].It is possible to date the first instances of malaria in human history. In the fourth century BC, Hippocrates recognized it as an illness. The Peruvian bark of the Cinchona tree was well-known for

treating fever in the early seventeenth century. Heinrich Meckel discovered granules of a black-brown pigment in the spleen and blood of a lunatic in 1847. Dichloro-Diphenyl-Trichloroethane (DDT) was created by Othmer Zeidler in 1874 for his thesis. In 1880, Alphonse Laveran discovered parasites in a malaria patient's blood that he named Oscillaria malariae. Ettore Marchiafava and Angelo Celli depicted the genus plasmodium in 1885. Ronald Ross first described the parasite's whole transmission cycle in 1897. The transmission of malaria to humans by Anopheles mosquitoes was proven in 1898 by Camillo Golgi and colleagues. Hans Andersag made the discovery of chloroquine in 1934. He gave his mixture the name resochin. Early in the 1950s, it was believed that malaria had been wiped out in the USA. Plasmodium knowlesi infection in humans was then discovered in 1965. In 1971, the herb Artemisia annua's artemisinin was isolated. The development of rapid diagnostic tests (RDTs) for malaria followed the depiction of PCR-based malaria detection in the early 1990s.

Dinoflagellates, which are photosynthetic protozoa, are assumed to have been the ancestors of the genus Plasmodium, which is the cause of malaria. At least 13 of the more than 200 distinct Plasmodium species are harmful to people. There are five of them that are well-known as the causes of human malaria: falciparum, vivax, ovale (two species), and malariae. Additionally, when a person is bitten by an Anopheles mosquito that has been infected by a monkey, knowlesi illness may strike. Of these species, falciparum (prevalent in East and Southern Africa) is most common on the African continent and is in charge of the majority of malaria-related fatalities. Geographically, Plasmodium vivax is more widely distributed. Although it may happen everywhere in Africa, the probability of contracting vivax there is quite low since many African people lack the Duffy gene. However, there is mounting evidence that vivax is spreading among people in Africa, notably Ethiopia, who do not have the Duffy blood group [3], [4].

DISCUSSION

Anopheles mosquitoes, which have more than 537 identified species, are the primary vectors of malaria transmission. The two most effective vectors for malaria in the globe are A. As well as G. funestus, are the main sources of malaria in Africa. A. Gabbiae, A. both funestus and A. Pharophora were determined to be the main vectors in Ethiopia. Due to the parasite's residency in RBCs, malaria may also be spread through blood transfusion, organ transplant, or by sharing needles or syringes that have been contaminated with blood. Congenital malaria may develop before or during birth in a newborn child. Furthermore, global climatic patterns like El Nino and La Nina have a significant impact on malaria transmission. Malaria poses a threat to around 44% of the world's population. According to the most recent estimates, there were 219,000,000 cases of malaria worldwide in 2017, with the most (92%) coming from the WHO African Region. There were also 435,000 fatalities due to malaria, with the majority (93%) occurring in the same region. Falciparum is the cause of almost all (99.7%) instances of malaria. Globally, 266,000 children under the age of five are predicted to die from malaria in 2017. Women who are pregnant are more vulnerable to falciparum malaria. Falciparum causes 8–14% of low birth weight in malaria-endemic regions, which reduces the likelihood that a baby will survive.

In 2017, the number of malaria cases worldwide decreased by 59%. This year, death rates decreased by 44.1%, according to reports. In addition to health-related effects, the condition places a heavy economic cost on society in the form of missed workdays. Of course, malaria is thought to reduce certain African nations' economic development by 1.3% and their public health spending by 40%. It has a variety of effects on developing nations, including a reduction in tourism.One of the top ten and most prevalent infectious illnesses in Ethiopia was malaria. Ethiopia has a population of 28,548,422 individuals that live in a region at high

risk of contracting malaria. 1,530,739 confirmed cases of malaria and 356 fatalities were recorded by Ethiopia's Federal Ministry of Health (FMoH) in 2017. Since 2010 Ethiopia has shown a decline in the incidence and mortality rates of malaria, although high prevalence was still seen in certain regions despite high household coverage of control programs. People with low socioeconomic position may be linked to this increase. Only 50 percent of Ethiopia's malaria development reduction goal has been attained. To achieve the sustainable development objectives, the nation must improve its malaria prevention and treatment strategies.

The Malaria Parasite Life Cycle

When an insect bites the skin, it explores for a blood vessel to feed from, releases different vasodilators to boost its probability of locating a vessel, and salivates into the blood to avoid clotting. This process transmits the motile infectious form of Plasmodium, called a sporozoite. The circulatory system transports the thread-like sporozoites to the liver within 30 to 60 minutes following injection. The sporozoites mature into schizonts during the course of 7–12 days, and they may produce up to 30,000 merozoites before they rupture the hepatocytes. However, some sporozoites from the vivax and ovale species develop into hypnozoites, a type that may stay dormant in the liver for months or years and result in relapses in infected individuals. Fascinatingly, falciparum malaria recurrence has been seen in individuals who left an endemic location some years before. It indicates that Falciparum sometimes enters a dormant state. The asexual cycle then starts with the merozoites encroaching on RBCs to feed on hemoglobin and develop. The parasite develops inside the host RBC from the early ring stage to the late trophozoite, and then, after mitotic divisions, to the schizont stage, which, depending on the parasite type, comprises 6 to 32 merozoites [5]–[7].

The discharged merozoites continue the life cycle by infecting additional RBCs after the erythrocytic schizont ruptures. Cyclical fevers often occur just before to or simultaneously with RBC lysis when schizonts burst to release fresh infectious merozoites. Infection with tertian malaria happens every 48 hours, while with quartan malaria, it happens every 72 hours. During this repeating cycle, some merozoites mature into erythrocytic gametocytes, which are single-nucleated male and female sexual forms, and wait for the arrival of a blood-seeking female Anopheles mosquito. The mosquito's consumption of gametocytes causes gametogenesis to occur. The macrogametes that produce zygotes are penetrated or fertilized by the exflagellated forms of microgametes. The zygotes develop into ookinetes before becoming a spherical oocyst. The nucleus inside the oocyst repeatedly splits, resulting in the development of many sporozoites and expansion of the oocyst. The sporozoites are released into the haemocoel the mosquito's bodily cavity when the oocyst bursts after they are completely grown. The life cycle is finished when the sporozoites move on to the salivary glands. The malaria life cycle is continued by the sporozoites from the salivary glands of the mosquito entering a new human host.

Malaria Pathogenesis and Cell Biology

Since Plasmodium is a eukaryotic microorganism, it is hypothesized that its cell biology is comparable to that of other eukaryotes. At the molecular and cellular levels, the parasite's whole lifecycle and pathology are not well understood, but. Since a thorough knowledge of the biology of the malaria parasite is necessary to participate in these activities, we have combined old and contemporary data here to enlighten and click specialists to include them in the development of vaccines, innovative medications, and malaria control methods. Rhoptries, dense granules, and micronemes are a group of apical organelles that distinguish

all Apicomplexa, including malaria parasites. There are three invasive forms of Plasmodium spp.: the sporozoite, the merozoite, and the ookinete, which uses the apical organelles located at one end of the parasite.

Through a process known as traversal, the sporozoite that was injected into the host epidermis first enters the bloodstream and then swiftly gains access to the liver. Review of the proteins required for traversal may be found in. The circumsporozoite protein (CSP) and thrombospondin (TSP) domains on sporozoites, for example, which facilitate invasion, have glycoaminoglycan chains that bind particularly to the heparan sulfate proteoglycans (HSP) on hepatocytes and Kupffer cells. Falciparum entrance and invasion in hepatocytes are shown to be mediated by at least two receptors, CD81 and CD68. Sporozoites travel through a number of hepatocytes after entering the Disse space of the liver before engaging in a final invasion that results in the creation of a parasitophorous vacuolar membrane (PVM). Merozoite egress from the diseased hepatocyte and PVM is then broken by plasmodial proteolytic enzymes to get access to blood circulation.

Merozoite surface protein-1 (MSP-1) attaches to the surface proteins of RBCs in the bloodstream through a glycosylphosphatidylinositol (GPI) anchor associated with the parasite membrane. There have been reviews of eight other merozoite surface-bound GPI-anchored proteins that interact with RBC elsewhere. An important entryway for the malaria parasite entering RBCs has recently been discovered to be CD55, a protein on the surface of RBCs. This finding offers up a fresh avenue for the creation of malaria treatment and prevention medicines. After attaching to RBC, the merozoite uses apical membrane antigen 1 (AMA-1) to realign itself such that the apical end of the parasite would locate next to the RBC membrane and cause a brief deformation of the RBC. As the parasite invades, the contents of the apical organelles will be ejected [8], [9].

Microneme proteins that identify and bind to receptors in the host recognize and create a connection between the parasite and host cell after reorientation and microneme discharge. The tight junction is created by inserting proteins from the neck of the rhoptry into the host membrane and binding them to AMA-1. RBC membrane proteins are then removed from the contact region. The RBC's submembrane cytoskeleton and lipid architecture are then locally disrupted by the merozoite enzyme serine protease. The release of the rhoptry bulb, which supplies the proteins and lipids needed for the parasitophorous vacuole, is triggered by the junction's formation. So, at the junction region, an incipient PVM will develop. The parasite seems to pass through this annulus as it enters the growing parasitophorous vacuole, where the interface between the parasite and host takes on the appearance of a ring. The PVM and host cell membrane close after the entryway.

The apicomplexan parasites that are invasive are mobile types that use "gliding motility" to travel over the surface. The parasite essentially creeps inside the host cell during invasion through the moving junction. In gliding motility, the organism must continually release its micronemes as it moves, and it must continuously build new connections between the zoite and the substrate. The inner membrane complex (IMC), which is located under the plasma membrane, would be home to a myosin exclusive to the Apicomplexa. Actin will interact with the IMC-associated myosin as a component of the glidesome. The moving junction (MJ) complex, which is made up of several adhesins, is subsequently connected to the glidesome.

It is possible for mature parasites to sequester themselves in a variety of organs, including the heart, lung, brain, liver, kidney, intestines, adipose tissue, and placenta, by adhering to the capillary and postcapillary venular endothelium in the deep microvasculature. This disease-related trait has only ever been linked to falciparum. It has furthermore been reported in

vivax-infected reticulocytes. PfEMP1 emerges on the surface of the infected red blood cells (IRBCs) around 16 hours after the invasion to attach to the endothelium.

When IRBC attaches to placental chondroitin sulfate A (CSA), which is mediated by Variant Two chondrotin Sulphate Antigen (VAR2CSA), sequestration is also seen during pregnancy. As a result, placental malaria may result in congenital malaria, intrauterine development retardation, low birth weight, and miscarriage. Adherence enables parasites to evade clearance by the spleen and to hide from the immune system, while sequestration gives them the microaerophilic venous environment that is more suited for their development. Red cell rosetting and agglutination are both formed when IRBCs attach to other parasitized RBCs as well as to uninfected RBCs. PfEMP1 has been shown to bind to the ABO blood group, complement receptor-1 (CR-1), and heparin sulfate (HS) during rosette formation. PfEMP1's lectin-like duffy-binding domain (DBL), in particular the blood group A antigen, may form a strong adhesion with carbohydrate structures. Because of their heightened rosette development, non-O blood types are risk factors for malaria that may be fatal. All three types of rosettes falciparum, vivax, and ovale can develop, although only those brought on by falciparum have been linked to life-threatening malaria. Young RBCs are clearly preferred by Vivax and Ovale, while Malariae favors older cells. These parasites thus have low parasitemia levels in the blood. However, Plasmodium falciparum may infiltrate RBCs of all ages and results in very high levels of parasitemia.

The aforementioned pathophysiologic process eventually stops blood flow, restricts local oxygen delivery, hinders mitochondrial ATP generation, and increases cytokine production, all of which contribute to the onset of a severe illness. Additionally, as MSP-1 that has been processed by subtilisin-like protease 1 (SUB1) interfaces with the spectrin network of the RBC cytoskeleton, the host RBC ruptures or lyses to allow parasite escape . Toxins (red cell membrane products, hemozoin pigment, and GPI) are also released into the blood along with the destruction of IRBC, activating macrophages and endothelial cells to produce cytokines and inflammatory mediators. The produced cytokines and poisons have been mostly blamed for the systemic symptoms of malaria, including fever.

Additionally, the Toll-like receptor-9 interacts intracellularly with the plasmodial DNA provided by hemozoin, causing the production of proinflammatory cytokines that in turn cause prostaglandins to upregulate COX-2 and ultimately cause fever induction. Hemozoin has also been connected to anemia-causing apoptosis in the bone marrow's developing erythroid cells. Mawson further proposed that the parasites enter the RBCs by emerging from the liver loaded with vitamin A and employing retinoic acid as a cell membrane destabilizer. This invasion results in hemolysis and anemia. The development of potent proinflammatory immune responses is strongly associated with the clinical symptoms of severe malaria, notably those produced by the falciparum parasite. One of the main causes of cerebral malaria vasculopathy is an overactive immune response, and fatal outcomes are often attributed to the sequestration of activated macrophages, parasitized erythrocytes, and platelets in brain arteries [10], [11].

Detection of Malaria

In order to treat patients effectively, it is essential to make an early and precise diagnosis of malaria. It may be broadly divided into clinical diagnosis and parasitological diagnoses. The patient's symptoms and the physical examination's findings serve as the basis for the clinical diagnosis. In every situation, a parasitological diagnostic should be performed to confirm any suspected cases of malaria. RDTs and light microscopy are frequently used techniques for parasitologically diagnosing malaria. The gold standard for diagnosing malaria is the light

microscopy detection of the parasites on giemsa-stained peripheral blood smears. Microscopy alone cannot identify knowlesi because it shares virtually all of its morphology with malariae. All phases of development following the hepatic cycle may be detected in the peripheral blood in the cases of vivax, ovale, and malariae. However, as mature parasites get sequestered in falciparum, only ring forms and gametocytes resembling bananas are often seen in peripheral blood.RDTs, which are based on the detection of antigens or enzymatic activity linked to the parasites, are a viable alternative to microscopy in places where it is not widely accessible. The two enzymes of the parasite glycolytic pathways, plasmodial lactate dehydrogenase (pLDH) and aldolase, as well as P. falciparum histidine-rich protein-2 (PfHRP2), which is specific for falciparum malaria, are the most often encountered antigens for RDTs. LDH may be a variation that is pan-specific (found in all six species) or unique to falciparum or vivax malaria. When adult parasites are isolated, RDTs may also assess parasite antigens.

However, several isolates from the Amazon area, Africa, and India have been discovered to lack the PfHRP2, most likely due to HRP2 gene deletion, endangering the capacity to identify and treat falciparum malaria patients effectively. Single-species RDTs were first made available in Ethiopia in 2005. Years later, FMoH is still providing multispecies RDTs to health posts. Although not a viable tool for everyday use, PCR-based methods, another kind of parasitological diagnostic method, are the most sensitive test able to detect low levels of parasitemia, parasite species, or mixed infections. For recognizing knowlesi infections, a technology known as loop-mediated isothermal amplification (LAMP) that is species-specific has gained widespread acceptance. Additionally, the use of PCR as a research technique is beneficial for epidemiological studies, clinical trials, and the identification of molecular indicators of antimalarial drug resistance.

The fourth parasitological approach uses an enzyme-linked immunosorbent assay (ELISA) or indirect immunofluorescence (IFA) to identify antibodies against malarial parasites. Serology evaluates prior exposure rather than present illness. Micromagnetic resonance reflaxometric (MMR) and rolling circle-enhanced enzyme activity detection (REEAD) tests, which were recently developed, are very accurate, cost- and time-efficient, and may be used in the field. Extremely conserved protein genes have been investigated as potential new malaria diagnostic targets. HSP-70, the most prevalent heat-shock protein (HSP), has been researched as a potential novel diagnostic protein. The Plasmodium heme detoxification protein (HDP) for all Plasmodium species, the protozoan dihydrofolate reductase (DHFR) for falciparum and vivax spp. detection, the glutamate-rich protein (GLURP), and the high-mobility group box 1 (HMGB1) protein for falciparum diagnosis are additional intriguing targets. According to a recent research, malaria-infected youngsters smell more enticing to the Anopheles gambiae mosquitoes that transmit the illness. Most of the change in attractiveness was caused by an increase in chemical discharges called aldehydes. The discovery may help in the development of cutting-edge noninvasive diagnostic techniques since it makes it possible to identify malaria parasite carriers using scents, even in people who do not feel ill enough to attend a hospital.

Vaccine for malaria

To accomplish the goals of the global malaria eradication campaign, a safe and effective vaccine is needed due to the establishment and spread of drug and insecticide resistance, which has been reducing the effectiveness of existing malaria control techniques. The development of a malaria vaccine is justified by the observation that residents of endemic regions develop clinically protective immunity despite the parasites' ability to evade the host's protective immune responses due to morphological changes and antigenic variations

throughout their life cycle. Pre-erythrocytic vaccines to prevent blood-stage infection, blood-stage vaccines to clear parasitaemia and prevent clinical disease, and transmission-blocking vaccines to prevent mosquito infection and stop the spread of malaria in populations have all undergone extensive research to date. Pre-erythrocytic vaccinations focus on the parasite's sporozoites and/or hepatic stages. Some vaccines in this class include RTS,S/AS01, which is undergoing a phase IV clinical trial, the falciparum sporozoite vaccine (PfSPZ), which is undergoing a phase II trial, the vivax malaria protein 1 (VMP001/AS01B), which is undergoing a phase I/IIa clinical trial, the cell-traversal protein for ookinetes and sporozoites (CelTOS), which is being Falciparum liver-stage antigens (PfLSA-1, 2 & 3) and vivax liver-stage antigens (PvLSAs) have been identified via in vitro research as a unique potential vaccination that targets infected hepatocytes.

There are a number of asexual blood stage vaccines in clinical trials, the majority of which target merozoite antigens. AMA1, erythrocyte-binding antigen (EBA-175), MSP-1 , MSP-119 , MSP-2 , MSP-3 , and serine repeat antigen 5 (SERA5) are candidates for the erythrocyte-stage vaccination. None have shown obvious clinical protection, most likely as a consequence of the vaccine structures' extreme polymorphism. But even though new nonpolymorphic falciparum ligands, CX3CL1-binding proteins (CBP1 and CBP2), are now revealed by Hermand and his coworkers, providing a new opportunity for innovative vaccination approaches , efforts to increase the efficacy either with a novel adjuvants , using viral vector prime-boost strategies , or by combining AMA1 and MSP1 have been increasing. New antigens, including rhoptry-associated leucine zipper-like protein 1 (RALP1) and falciparum reticulocyte-binding protein homolog 5 (PfRH5), may one day serve as candidates for blood-stage vaccines. Actually, a phase I clinical investigation of PfRH5 is underway. There is now interest in include falciparum merozoite protein MSP4 in candidate vaccines since it naturally elicits a potent antibody response in malaria endemic regions. After one pregnancy, multigravidae who have developed antibodies against VAR2CSA are in fact shielded against malaria caused by pregnancy. Vaccine candidates against VAR2CSA are being improved in light of this discovery.

By specifically targeting host antibodies, complement proteins, and cytokines, transmission-blocking vaccines (TBVs) limit the development of parasites in the midgut of mosquitoes by targeting surface proteins expressed on gametocytes, zygotes, and ookinetes. The gametocyte antigens (Pfs48/45 and Pfs230), the falciparum ookinete surface antigens (Pfs25 and Pfs28), and their vivax homologues Pvs25 and Pvs28 are among the vaccination candidates in this category. Pfs47, which is involved in parasite immune evasion in the mosquito vector, and PfHAP2, which is expressed on the male gametocyte and microgamete, are two additional more recently discovered targets of intrigue. Within the three vaccination categories stated above and beyond the purview of this page, several vaccines with formulation issues are covered in depth elsewhere.

CONCLUSION

In endemic nations, malaria, an old disease of humans, continues to be a major source of sickness and death in both children and adults. Malaria caused by vivax and falciparum poses a significant threat to public health. Malaria prevalence and incidence may be declining, but the disease's transmission is still active worldwide. As a result, controlling malaria necessitates an integrated strategy that combines vector control as the primary form of prophylaxis with quick antimalarial therapy. A concern in the battle against malaria is the development of resistance to preventative measures and the antimalarial medications that are already on the market. There are currently no approved malaria vaccines available after decades of intensive study. Despite the fact that numerous medications are under

development, the majority of them cannot kill both gametocytes and hypnozoites. If the history is any indication, Ethiopia will see resistance to traditional antimalarials in Africa. The malaria landscape is changing due to success and resistance, necessitating new instruments and methods. Therefore, the world urgently needs innovative, risk-free, and potent medications and vaccinations that can replace the existing phenomena of increased resistance. For the malaria eradication effort to go ahead in Ethiopia, more work must be done.

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CHAPTER 2

AN OVERVIEW OF MODELING, CONTROL AND MALARIA TRANSMISSION MECHANISMS

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ABSTRACT:

Malaria is a major source of illness and death in sub-Saharan Africa. The management of this condition might be difficult due to the interaction of several variables. Few studies have, however, shown the complexity, modeling, and management of malaria, despite the possibility that this viewpoint may result in sensible policy suggestions. This essay seeks to serve as a didactic resource that gives the reader an overview of malaria. More significantly, by using a system approach lens, we want to draw attention to the hotly contested issues and the many thematic facets of malaria transmission mechanisms, as well as the control strategies used and the model underpinning the dynamics of malaria. We tried to present an overview of malaria that has to be further expanded since there is a lot of material available on each topic. However, this research highlights the need of using a multidisciplinary approach when developing future malaria control strategies.

KEYWORDS:

Disease, Malaria, Mosquito, Malaria Transmission.

INTRODUCTION

Around 90% of malaria victims are found in sub-Saharan Africa (SSA), where almost 2000 youngsters each day perish from the disease. Complex interactions and feedback loops between people, mosquitoes, parasites, their habitats, healthcare systems, and the adoption of policies at a particular point in time influence the transmission and management of malaria. As a result, it is possible to characterize malaria transmission as complex, nonlinear, and dynamic. For instance, the endemism of malaria in West Africa has significant financial effects. One malaria episode in Ghana may cost up to 34% of a household's annual income to cure. The danger of contracting malaria rises as a consequence of infected persons being unable to generate money and as wealth is low. Spending on malaria prevention has a negative correlation with national economic development. As a result, malaria may be seen as both a contributing factor to and an effect of poverty. The nonlinear nature of malaria infection was not taken into account by earlier therapies, which is another reason why they were ineffectual. Particularly, the unanticipated effects of treatments, such as parasite and mosquito medication resistance, resulted in their withdrawal. This was the case with the global malaria control strategy that was implemented in 1992 and the eradication control effort that was discontinued in 1969. The complexity of malaria has been well accepted by scientists, but a representation of this complexity is poorly supported, which further restricts the reach of malaria management [1], [2].

This concise study covering a number of subjects or themes gives the reader a foundational understanding of malaria transmission, control, and modeling while highlighting its complexity. The text aims to be a didactic piece, giving a succinct summary of the illness,

eliminating information asymmetry, and piqueing readers' interests so they may consult more specialized literature.We only looked for peer-reviewed articles on malaria between 1950 and 2018 in the online scientific database. The first malaria model was created in the 1950s, and since then, most of the suggestions for malaria prevention have been backed up by quantitative research. This provides the justification for this time range. Additionally, we eliminated any duplicate letters, case reports, narrative reviews, and comments. We restricted the scope of the study to just peer-reviewed, published studies in either French or English due to the extensive research on malaria. In order to minimize biased translation and potentially difficult to repeat findings, this was done. We do not include any case reports within the subject of interest that occur in non-endemic regions.

PUBMED, Google Scholar, and MEDLINE were the three databases we examined to find relevant articles on malaria published between 1950 and 2018. Our screening procedure is centered on the study's title as it appears in peer-reviewed publications. The research that did not fall within the keywords were removed once we had reviewed the abstracts of the publications. We divided the list of 77 suitable peer-reviewed publications into relevant subjects of interest, such as mechanisms, prevention and therapy, mathematical modeling, and views on modeling, in order to get a thorough grasp of malaria. We were able to get a comprehensive understanding of the state of the art thanks to the thematic structure of the gathered articles [3], [4].

DISCUSSION

The search yielded 2,150,000 studies. After eliminating duplicates, 150 000 studies were retrieved in total. Following a title and abstract screening, we only included 300 research. Additionally, we eliminated 16 papers, including 10 thesis and 6 NGO reports, 146 research conducted in malaria-free regions, and 50 case reports. We summarized each subject using the remaining 77 appropriate research and added 10 more readings to our narrative as well as WHO and CDC data. There were 12 papers on upcoming research on malaria modeling, 19 studies on prevention and therapy, 23 studies on mathematical modeling, and 23 studies supporting malaria transmission.

Mechanism of Malaria Transmission

Humans, mosquitoes, and the pathogen interact to generate malaria infection. When female mosquitoes lack the protein and plant sucrose needed for egg development during the egg-laying phase, they undergo a transformation. Since they feed on human blood, they may infect people by biting them and injecting infectious protozoans (of the species Plasmodium) into the circulation. The Anopheles Giles genus contains the majority of female mosquitoes in West Africa. Sensu stricto, Anopheles gambiae prefers to consume human blood to finish its gonotrophic cycle, bites mostly inside (endophagic), and lays down indoors (endophilic). With regard to its feeding habits, An. The primary malarial vector in West Africa is the gambiae. There are also more vectors, such as An. Arabicus, A. and Funestus. P. falciparum-carrying melas. Comparable to An. the gambiae, An. Funestus exhibits endophagy, endophilia, and anthropophilia. As opposed to that, An. Exophilic, exophagic, and zoophilic behaviors are all shown by arabinoses. It loves blood-feeding animals and bites and rests outside. An. Additionally, melas exhibits anthropophagy, exophagy, and exophilia. As a result, P. falciparum adapts well to Anopheles species, making the host more susceptible to malaria. Malaria modeling is made more difficult by the large variety of vector circumstances, dietary preferences, and geographical variability in malaria transmission.

A mosquito's sensitivity to environmental factors like humidity and temperature affects how well it can adjust to those circumstances. This modification may modify the duration of the

life cycle or the effectiveness of the bite. The feeding habits and efficiency of vectors may alter with time, as a lengthy research conducted in Kenya (1999–2010) has shown. Because vectors' attraction to the host changes, the transmission strength may fluctuate dramatically between host populations. The variance in human body microbiota and responses to mosquito bites are the causes of this variation. For instance, compared to a host that isn't affected, an infected host is more attractive to other mosquitoes. Similar to humans, mosquitoes choose the aroma of kids over that of adults [5]–[7].

Plasmodium vivax, P. ovale, P. malariae, P. knowlesi, and P. falciparum are infectious protozoans found in SSA; P. falciparum is responsible for 95% of malaria cases. The whole life cycle of P. falciparum includes an incubation phase lasting 10 days in the vector and a phase lasting 7 to 20 days in the human host. The P. falciparum protozoan completes its life cycle when it is introduced into the circulation of a host via the saliva of the vector. The sporozoites (asexual protozoans) travel into the liver after entering the host's circulation and then pierce the hepatocytes (liver cells). The parasite undergoes schizogony, or multiple asexual divisions, in the host hepatocytes. When the hepatocytes eventually rupture, merozoites are released into the circulation of the host and infest red blood cells. After several divisions of the red blood cells, the parasites are capable of diverging into gametocytes. Before and during the differentiation of P. falciparum into sexual phases, malaria may produce symptoms including fever, chills, and sweating. Even yet, some hosts could be categorized as asymptomatic since they don't show any clinical signs. Mosquitoes predominantly sucking out the gametocytes when they bite infected persons (symptomatic and asymptomatic). In endemic locations, it might be difficult to identify asymptomatic people, and mass testing may not always be an economical approach to do so.

Malaria Treatment and Prevention

Existing control measures that target either mosquitoes or parasites may not be successful because of the recursive interactions between people, insects, and the parasites that promote malaria transmission. Mosquitoes have mostly been managed by eradicating their larval and adult stages while lowering the rate of entomological inoculation. The environment of the vector may be altered physically, biologically, or chemically as one of these controls. Breeding areas are eliminated as part of the physical change, and drainage and weeding are used to control the sources of larval. Although it is one of the most successful, this approach requires a regular workforce for weeding and drain cleaning, which caused SSA governments to abandon it. Application of larvicides and insecticides is one method of chemically altering the environment for larvae. The drawbacks of this approach include expense and the frequent development of insecticide-resistant mosquitoes. The biological approach makes use of mosquito larvae's natural enemies, including fish that feed on larvae and microorganisms (such as Bacillus thuringiensis). However, the short-term persistence of natural enemies interferes with this process.

In order to reduce adult mosquito populations in Africa in the 2010s, the Global Malaria Action Plan (GMAP) and the World Health Organization (WHO) advised using indoor residual spray (IRS) and insecticide-treated bed nets (ITNs). Synthetic pyrethroid, a deadly substance that kills mosquitoes, persists in the environment, and is safe for mammals to consume, is the primary chemical component of ITNs and IRS. Due to the effective use of ITNs and IRS, malaria cases in Ghana decreased by 41% between 2005 and 2010. Mosquitoes have had to adapt to the lingering effects of synthetic pyrethroids in the environment brought on by IRS, ITNs, and agricultural pest control. These consist of early biting, interior and outdoor spray avoidance mechanisms, and pesticide resistance.

Three methods of prevention vaccination, seasonal prophylaxis, and antimalarial medications are used to control the parasites that cause malaria. The Artemisinin-based Combination Therapies (ACT) are GMAP's first line of therapeutic measures. Compared to other curative therapy, ACTs had a greater clearance rate and symptom resorption against P. falciparum. ACT resistance has however been gradually acquired in P. falciparum. The use of fake malaria medications in SSA exacerbates parasite resistance to malaria. Another preventative strategy is intermittent prevention, which entails giving pregnant women a single dosage of malaria medication. The combination of primaquine, sulfadoxine-pyrimethamine, amodiaquine, methylene blue, and dihydroartemisinin-piperaquine, all of which have been shown to be effective in stopping the spread of P. falciparum, is used in the therapy. With an effectiveness rate of 82%, the seasonal vaccine "RTS, S/AS01" has been shown to be safe and efficacious for children under the age of five. The researchers showed that RTS, S/AS01 triggers an immunological response to the infected sporozoite proteins [8]–[10].

Clinical malaria is protected against by vaccine components. With time and aging, the efficiency of its preventive function may diminish. The vaccination is more beneficial for infants between the ages of 6 and 12 weeks, as opposed to those between the ages of 5 and 17 months. The use of phytotherapy as a malaria treatment is a less common practice. For instance, a research on phytochemicals in central Africa found more than 20 native plants and herbs that may treat and stop the spread of malaria with nearly little danger of P. falciparum acquiring resistance to these organic compounds. The author’s claim that a variety of chemical compounds in plants interact synergistically to cure malaria holistically while reducing the development of resistance.

Malaria Mathematical Modeling

Past and contemporary actions to prevent or cure illnesses have been impacted by mathematical models created to obtain insight into disease transmission. In 1911, the Susceptible-Infectious-Removed (SIR) model, which divided the population of hosts and vectors into three categories, marked the beginning of mathematical modeling of malaria transmission. The compartments were identified as susceptible (S), which stood for the population most prone to get the disease, infectious (I), which represented the portion of the diseased population, and removed/recovered (R), which represented the portion of the population that either passed away or recovered from the illness. The SIR model used a number of assumptions, such as that the population was closed and limited that the rate of mosquito bites was homogenous, and that the population was well-mixed. Due to its poor prediction, the Ross model (SIR) was unable to adapt to the new incidence data, but it nevertheless sheds light on the complex interaction between infected hosts and mosquito concentrations. Ross's theoretical and simulation-based approach was unable to provide evidence to support mosquito eradication programs since it was just theoretical.

The SIR model was then enhanced by George Macdonald by adding actual data to the model and incorporating an extra compartment for the latency interval designated Exposed (E) between the mosquito bite and the start of symptoms. Additionally, Macdonald's work supported the idea of a widespread effort to eradicate mosquitoes on the grounds of superinfection. So, as part of the Global Malaria Eradication Program (GMEP), the WHO carried out extensive and rigorous mosquito eradication programs between 1955 and 1969. Major European and American nations utilized the insecticide Dichlorodiphenyltrichloroethane (DDT) to effectively combat malaria during these campaigns. However, owing to mosquito resistance, the GMEP was unable to completely eliminate malaria. In addition to mosquito pesticide resistance, the lack of essential healthcare facilities, the high intensity of malaria transmission, and other socio-ecological variables

made it difficult for eradication programs to be successful in the SSA [11], [12].The mathematical epidemiology of malaria has progressively advanced over the last several decades, moving from "toy models" which were not realistic but caught the essential aspects of the illness to "high-level models" which are accurate but lose generality. As a result, complex models share traits including the interaction of several components. For instance, an earlier partial differential model in the SIR framework divided the infected population into age-dependent illnesses. The reproductive number (R0), which describes the spread of a disease, is a crucial measure. In a population that is completely susceptible, R0 reflects the anticipated number of infected human hosts. Thus, when R0 is more than one (R0 > 1), it helps define locations where a disease is endemic and offers a gauge for the intensity of transmission. Therefore, heterogeneity was taken into account while calculating R0 in populations. These include, for instance, the host's age distribution, the vectors' and hosts' migration, the host's beliefs and behaviors, and the host's socioeconomic classes. The intricacy of the population compartmentalization is another factor that affects R0. The number estimated for R0 becomes closer to actual transmission dynamics the more the modeling framework captures the variability in the host population. Maximum control has scarcely been attained in, and the pioneering models that shed light on malaria dynamics and its controls were impractical for a number of reasons. In particular, a large R0 indicates a greater mosquito density, which diminishes the efficacy of ITN in a sizable and probably varied host population.

There are several mathematical models that include the compartments S, E, I, and R and take into account population heterogeneity (meta-population modeling techniques). Examples include SIR, SIS, SI, SIRS, SEIR, SEIRS, and SEI. Due to their propensity to assume vast, homogenous populations that ignore the presence and unique properties of subpopulations, the majority of these models fail to represent the dynamics of subpopulations. Another problem is that they neglect to take into consideration host behavior, which affects the dynamics and management of the illness. Additionally, compartmental models (like the Ross model and Macdonald models) are either knowledge- or data-driven. The intricate interactions between factors that control the development of the illness in both situations are beyond the scope of the models. For instance, socioeconomic factors affect the variables that are often taken into account when calculating R0, such as mortality, mobility, and birth rate. Models that accurately depict the malaria transmission mechanism are therefore still lacking.

Modeling Malaria: Current and Future

The development of computational power and the availability of data improved the capacity to follow epidemics. Although the deterministic paradigm used in this setting offers insight into disease processes, it often overlooks uncertainty. On the other hand, stochastic models take randomness that could happen during the epidemic into consideration and, more crucially, account for a wide range of uncertainty that happens throughout epidemics. For instance, a stochastic model was used to forecast the intricate pattern of transmission for environmental changes. Similar to deterministic models, stochastic models provide insights into the rate of the spread of vector-borne diseases in a new area in the context of climate change as well as other interesting aspects of the transmission process, such as the influence of network structure and the characteristics of an outbreak . Additionally, both deterministic and stochastic models are population-based and often overlook the characteristics of individuals, in contrast to individual-based models (IBMs), which do [13], [14].

Individual agent heterogeneity and spatial gradients are both taken into consideration by IBMs. However, parametrizing them continues to be a difficult endeavor, particularly given the scarcity and reliability of data. Within-host modeling, which goes beyond meta-

population modeling (Ross-based models), has created new avenues for addressing malaria infectiousness and exploring the interaction between host immune systems and parasite dynamics. However, it is still very difficult to simulate the superinfection of malaria while keeping track of each parasite strain and taking into account the various genetic composition of the parasites. Most recently, the area of epidemiological monitoring was improved by the introduction of a new form of data, such as human mobility obtained from data providers (Facebook, Twitter, Google, etc.). These information has been utilized to identify malaria hotspots and forecast the spread of the disease. The modeling and documentation of evidence for policy recommendations is made possible by the use of fine-grained mobility data, but privacy issues must be addressed.

Despite tremendous study, malaria is still a significant health issue in Sub-Saharan Africa. The behavior of the human host and vector as well as socio-ecological conditions and potential genetic alterations in the parasite all play a role in the transmission of malaria. Modeling malaria involves several difficulties due to its complexity. For instance, due to a dearth of trustworthy historical data, it might be difficult to appropriately parameterize a model with a compartment of asymptomatic people. Evidence, however, points to the possibility that malaria symptoms might develop quickly, going from nonexistent to life-threatening, while also leaving some patients with a lifelong weight of cognitive deficits. Therefore, a method for producing trustworthy primary data on asymptomatic individuals with the potential to detect infectious Plasmodium at a cheap cost is required. The study also made clear that there are a variety of variables that may be explored for each of the themes covered, including employing a multidisciplinary lens to look at clinically important malaria concerns.

CONCLUSION

A historical perspective was utilized to show how the malaria model developed from simple to complex models. This paper also discusses modeling difficulties, including how adding mobile data to a mathematical framework can result in re-identification issues. Model parameterization is difficult due to a complicated modeling approach's large number and scale of parameters. Finally, this review reveals that controlling malaria effectively requires integrating a number of approaches. Constant awareness and education initiatives in at-risk groups and among people whose resistance to non-pharmaceutical therapies (such ITNs) might obstruct malaria control efforts are a few of them. We admit that we have only touched the surface of the different cross-cutting subjects given the broad variety of thematic areas mentioned. Since these topics are extensively discussed elsewhere, we did not go into detail on the host immunological response to malaria or about technical advancements in detecting and treating malaria. This work also has two issues: (1) a self-selection bias that might cause it to place greater focus on certain cross-cutting issues, and (2) not taking into account newly released studies. However, readers will still get a broad understanding of malaria from this article.

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CHAPTER 3

VISCERAL LEISHMANIASIS DIAGNOSIS ADVANCES IN THE ERA OF ELIMINATION

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ABSTRACT:

The most severe parasite illness in the world, visceral leishmaniasis (VL), has a significant morbidity and fatality rate. Asymptomatic or subclinical infection to severe and complex symptomatic illness are all possible clinical presentations of VL. The fragility of healthcare infrastructures in areas where the illness is prevalent is a significant barrier to clinical treatment of VL. Most VL patients visit primary health care facilities (PHFs) at the end of their treatment plan. PHC doctors deal with patients who approach with concerns that might lead to a number of different diagnoses, which is a significant difficulty. When individuals with fewer clinical signs get the wrong diagnosis, there is risk. Therefore, for efficient management and achieving the aim of VL eradication, field-based fast diagnostic techniques that are accurate, sensitive, and reasonably priced are crucial. In this review, we examine the use of several diagnostic methods for the diagnosis of VL, describe their present status and difficulties, and evaluate how well they work in situations with limited resources.

KEYWORDS:

East Africa, Health Care, Parasite, Visceral Leishmaniasis (VL).

INTRODUCTION

With an estimated global prevalence of between 0.2 and 0.4 million new cases each year, visceral leishmaniasis (VL), often known as kala-azar, is one of the most neglected diseases associated with poverty. Only six nations India, Bangladesh, Sudan, South Sudan, Ethiopia, and Brazil account for around 90% of these instances. L is the cause of VL in East Africa and the Indian subcontinent. donovani, which is caused by L. donovani throughout Europe, North Africa, and Latin America and is spread by the sand fly P. argentipes without any known animal reservoir. Chagasi (syn. L. infantum) have reservoirs in both humans and dogs. India alone has more than 100,000 occurrences annually, with the bulk of these cases occurring in the state of Bihar. These numbers, which are based mostly on passive case reporting and are regarded to represent an underestimate of the actual number of VL cases, are still official reports. VL is unmistakably a disease associated with poverty since it not only affects the lowest of the poor but also prevents the economic growth of the afflicted communities. Thakur examined the socioeconomic circumstances of a cohort of 938 VL patients from Bihar, India, in the year 2000. 82% of them worked in agriculture and/or animal husbandry, and 75% of them were considered poor (daily income US $1). Recently, VL has attracted the greatest public attention as one of the world's most neglected illnesses. Through a variety of control methods, the three nations on the Indian subcontinent (ISC) that are impacted by VL India, Nepal, and Bangladesh aspire to eradicate the disease from the region by the year 2015 [1], [2].

Reduced transmission by early identification and thorough treatment is one of the key goals of this effort. The positive predictive values of the diagnostic tests, however, decline with a higher chance of false positives when the control measures are successful in reducing the incidence of the targeted ailment. Therefore, it's crucial to check if the platform and format of diagnostic technologies are suitable for the level of infection prevalence in the specific area. The primary problem with control techniques is their long-term viability. Since primary care providers are the only ones capable of providing passive case discovery on a big scale and over the long term, primary care settings will eventually need to be re-involved.

However, in VL endemic regions, clinical choices are serial and dichotomized, which means that for a specific condition (for example, fever), the potential diagnoses are investigated step by step and using a "yes/no" approach. Therefore, a particular illness is taken into consideration when certain signs and symptoms are present, and when they are lacking, the disease is disregarded and a different diagnosis is sought. VL has a lot of complicated characteristics, and its clinical symptoms are often mistaken for those of other febrile infections. Patients who exhibit less clinical symptoms run the risk of receiving the incorrect diagnosis, which may delay receiving treatment and result in patient mortality. Since antileishmanial medications might result in serious side responses, a quick and precise confirmatory diagnostic test is required to address this genuine problem in clinical care settings.

The capacity of nations to assess the precise illness burden will be significantly impacted by reliable diagnostic techniques. Additionally, it would enable them to monitor illness patterns over time and assess the efficacy of upcoming control measures, such as enhanced diagnosis-treatment algorithms and fresh vector management techniques. The following sections provide a short overview of the existing diagnostic methods for VL, together with information on their efficacy and potential drawbacks in primary healthcare settings in disease-endemic regions [3], [4].

VL's Present Diagnosis and Challenges

The chronic febrile condition that VL is known for is often accompanied by splenomegaly and progresses to cause wasting, anemia, and mortality from bleeding or secondary bacterial infection. Control of this illness depends heavily on early discovery and appropriate care. The way VL is handled in endemic areas might be greatly impacted by noninvasive fast tests that are utilized at peripheral health clinics to identify VL and/or as a marker of cure.

DISCUSSION

The first line of defense in the diagnosis of VL is parasite detection, which is a highly specialized technique. The first-line noninvasive test is the microscopic inspection of a peripheral blood smear or buffy coat. The most precise technique of diagnosis is still in use in the Indian subcontinent and East Africa and uses splenic biopsies or bone marrow in the event of a negative result. Brazil has the highest prevalence of parasitological tests using bone marrow aspirates. These operations are not viable at the PHC level because to their discomfort, probable risk, and high degree of competence. Tissue aspirates or blood cells may be cultured in vitro with up to 100% sensitivity, but these procedures are costly, time-consuming, and laborious and can only be used in specialized research labs. Importantly, the only confirming test available for individuals who have had a recurrence is a parasitological test. Antibodies conjugated with fluorescent against the parasite's surface antigens have been used in endemic situations in Brazil, Spain, Tunisia, Italy, and Iran to improve the sensitivity of parasitological diagnosis. The use of the IFAT test is only permitted at referral hospitals due to the need of a fluorescence microscope.

Immunological Diagnosis: Quick and Non-Invasive

The identification of leishmania antigens or antileishmanial antibodies in blood samples forms the basis for immunological diagnosis. In order to replace parasitological techniques, a number of serological tests for VL have been developed and tested in various endemic areas with varying degrees of success. These tests' sensitivity and specificity depend on the antigens they use, and among them, the direct agglutination test (DAT) and rk39 ELISA have both undergone substantial validation in endemic regions to identify L. suggested for VL control strategies and donovani infection. However, the VL policy in Kenya mandates that all cases of leishmaniasis with a serological confirmation be verified by spleen aspirate, a process that can only be carried out at referral institutions.

DAT, or Direct Agglutination Test

The DAT is a semi-quantitative test that is widely used in field settings. It employs a microplate with a V-shaped well in which patient's serum or blood is serially diluted with coomassie-stained whole promastigotes antigen. After 18 hours (overnight incubation), agglutination may be observed with the naked eye if appropriate antibodies are present. DAT has received validation in a number of nations, including Brazil, Ethiopia, Kenya, Bangladesh, India, Nepal, and Sudan. The DAT has a sensitivity range of 70.5 to 100% and a specificity range of 53 to 100%, respectively. Strong correlations between the development of clinical VL and seropositivity or seroconversion with high DAT titer were discovered in a recent longitudinal research from India and Nepal. Overall, DAT's effectiveness as a diagnostic test is commendable, affordable (US $1.5-2.5), and independent of geographic location. Such diagnostic procedures cannot be carried out at PHCs because to the necessity for power, the storage of antigens at 2–8°C, repeated pipetting, and the need for qualified people [5]–[7].

Antileishmanial antibodies are often detected by ELISA, and the antigen employed is largely responsible for their sensitivity and specificity. In the past, ELISA was utilized with crude or soluble antigens of promastigotes or amastigotes; nevertheless, because of cross reactivity, it was given the lowest importance in diagnosis. Technology advancements have led to the development of numerous recombinant antigens for the diagnosis of VL, with rK39 ranking as the most effective (sensitivity: 67-100%; specificity: 93-100%). However, new generation fusion antigen k28 was created with enhanced sensitivity (92-100% in Sudan) owing to its poor sensitivity in Africa, without any alterations to its sensitivity in the Indian subcontinent. In Brazil, rK28 ELISA is also helpful in the diagnosis of cutaneous VL. The most significant limitation of all antibody-based detection methods, including ELISA, is that antileishmanial antibodies continue to exist for up to 16 years after the end of therapy and cannot be used to determine whether a patient has been cured or has relapsed . Another drawback of ELISA is that it can only be used in research facilities or well-equipped hospitals, making it impractical for use in endemic regions' outdoor settings.

RDT, or rapid diagnostic test

The recombinant K39 protein antigen-based RDT is readily accessible, repeatable, affordable (around US $1.0 per test), simple to use, and capable of providing results in 10 minutes. In the majority of the endemic locations, the rK39 RDT has good sensitivity and specificity. As a positive rK39 RDT in a healthy patient is not indicative of acute illness, its use is currently advised in conjunction with a clinical case definition. A significant downside of the fast rK39 strip test in India is that 15–32% of healthy people living in the endemic area test positive. As a result, non-VL patients with mimicking diseases such malaria, enteric fever, and other conditions may get antileishmanial medication. The rK39 RDT has been chosen as the

primary instrument for the VL eradication program in India, although its shortcomings are quite apparent. In East Africa (Sudan and Ethiopia), the response of the rK39 RDT exhibits sensitivity values between 70% and 94%.

In South America (Brazil and Venezuela), where sensitivities and specificities range from 86 to 100% and 82 to 100%, respectively, the rK39 strip test performs modestly. Recently, WHO evaluated five alternative immune-chromatographic tests (ICTs) using either rK39 or rKE16 in East Africa, Brazil, and the Indian subcontinent. The study's findings indicated a sensitivity range of 36.8 to 100% and a specificity range of 90.8 to 100%. No test stood up as the obvious victor across all areas and circumstances, however rk39 RDT demonstrated good diagnostic accuracy in India and Nepal. Later, the performance of the k39 RDT on serum and blood was compared in two independent investigations conducted in India, and both studies found good agreement and high diagnostic accuracy. Importantly, doctors continue to depend on clinical diagnosis in settings where K39 dipsticks are not easily accessible and parasitological diagnosis (such as spleen aspiration) is not practical, leading to overdiagnosis and inappropriate resource allocation. However, these RDT tests are worthless for diagnosing relapses or as prognosis (cure) tests since they cannot distinguish between present, subclinical, or previous infections.

More recently, the sensitivity of the rK39 RDT was tested and assessed using urine samples from Bangladesh and India, with sensitivity values of 96.1-100% and 95%, respectively. With saliva samples from Tunisia and India, the rK39 RDT also demonstrated excellent diagnostic performance. A test of the rK39 strip test's performance in HIV-positive and parasitologically confirmed VL patients revealed 77% sensitivity. To develop the rK39 RDT for the diagnosis of VL using saliva or urine samples and make it useful in the field for the detection of HIV-VL coinfection, further in-depth investigations are required [8], [9].

Test for Latex Agglutination (KAtex)

Theoretically, an antigen detection test would be regarded as more accurate than serological assays based on antibodies. In order to diagnose symptomatic infections in immunocompetent and immunocompromised individuals (such as the diagnosis of initial VL in Sudan, where rK39 RDTs lack sensitivity; the identification of recurrent cases) and to determine whether a patient has been cured, antigen detection diagnostic techniques are necessary. The effectiveness of the KAtex test to identify antigens in urine also serves as evidence that antigenaemia, an expected characteristic of VL, exists. The only diagnostic test that has been developed for the detection of 5-20 kDa glycoprotein in the urine of VL patients that is commercially accessible is called KAtex. KAtex is a simple, quick, and field-applicable test with a high specificity; nevertheless, in the investigations carried out in the Indian subcontinent and East Africa, the sensitivity was subpar and inconsistent. Although KAtex has promise as a predictive test and is 85.7% sensitive in HIV-VL coinfected patients, it has a significant drawback in immune-competent people. The noninvasive nature of KAtex makes it more acceptable in nonsymptomatic persons and would enable a longer follow-up. Urine can also be collected more readily than blood. The use of KAtex is restricted in outlying health facilities because to its limited sensitivity and necessity to boil the urine for five minutes to prevent false positive results, which impairs the test's repeatability.

Whole blood testing for leishmaniasis

The delayed type hypersensitivity response is measured by the Montenegro test or leishmanin skin test (LST). In the case of cutaneous leishmaniasis (CL), when healing lesions are largely evident, it is a highly helpful test. In the case of VL, it is often employed in endemic regions in conjunction with serological markers. It is very helpful in VL epidemiology even though it

has very little diagnostic value in VL (usually negative owing to anergic condition) . L-grade GMP. Donovani antigen is not accessible, and it is impractical to administer intradermally followed by a 48-hour test reading at PHCs. As a replacement for the LST, whole blood IFN-release assays (IGRA) have recently been developed, although their therapeutic use in the diagnosis or treatment of VL is not yet entirely demonstrated.

Specific and Highly Sensitive Molecular Diagnosis

Despite the fact that there has been significant advancement in science over the last ten years, including the sequencing of the L. Donovani, they have so far had little effect on the quality of clinical treatment provided for VL in the area. The cost and therapeutic utility of using molecular diagnostics in settings with limited resources is still up for dispute, despite the fact that their diagnosis accuracy is good in laboratory-based studies. Numerous molecular detection techniques that focus on certain DNA and/or RNA genes have been developed so far. The fastest and most sensitive gene amplification methods are polymerase chain reaction (PCR) and real-time quantitative PCR (qPCR), although these methods cannot be used in field situations. Additionally, relatively few tests have been verified on a variety of clinical samples, and none of them have emerged as standard diagnostics for VL. However, it is still unclear how these cutting-edge tools may be usefully used within the framework of the health systems in VL endemic regions. Many experts believe that these molecular tests will be crucial in ensuring the sustainability of the control program by identifying the infection at very low levels if the VL control program is successful in reducing the prevalence of infection [10], [11].

The New Challenge of Asymptomatic or Subclinical Infection Diagnosis in Endemic Areas

The fact that only sick persons will get treatment limits the effectiveness of chemotherapy alone as a VL control technique. Asymptomatic carriers make up likely parasite reservoirs for the sand fly vector, with ratios of 1:2.4 in Sudan, 4:1 in Kenya, 5.6:1 in Ethiopia, 18:1 in Brazil, 50:1 in Spain, 4:1 in Bangladesh, and 8.9:1 in high-endemic villages in India and Nepal (reviewed in). A new mathematical model contends that these asymptomatic infections play a significant part in the spread of human VL in ISC. Studies in which parasites could be cultivated from the blood of healthy donors or discovered by PCR provide compelling evidence that these infections are genuine. In order to locate potential hotspots of transmission in endemic regions, diagnostic testing for asymptomatic illnesses are required. There is no gold standard since using invasive techniques on asymptomatic people to show that they have parasites is immoral. Serological assays based on DAT or rK39 have been utilized in some research to record infection, and seroconversion for one or more tests has been employed in other investigations as a marker of infection.

Currently, there are numerous approaches to determine if an infection is asymptomatic: culture positivity, PCR and serology positivity, or a cellular immune response marker like the IGRA test. Moreover, when used cross-sectionally on the same demographic group, there is limited agreement across the various tests. The general assumption is that serologic testing (such as DAT, rk39 ELISA, and western blot assays) can identify recent infection, but it is unclear how long serology stays positive and whether it differs between VL patients and subclinically infected people (as seems likely based on the magnitude of the titers). It is challenging to determine whether these seropositives who stayed healthy were indeed L-infected in the absence of a gold standard. donovani, whether the serology findings were just false positives or an earlier infection that was successfully treated. Other elements, such as handling variability and storage procedures, may also have an impact on the robustness of

these test measures. Although L. is typically assessed with serological testing. Donovani infections, there is little information that has been published on how reproducible they are when used on asymptomatic people.

The only reliable method to determine the infectivity of such silent illnesses in disease transmission is xenodiagnosis. Xenodiagnosis was a technique employed by Molina et al. to identify VL infection in HIV-positive individuals. Sand flies might get HIV infection from even asymptomatic individuals in the early stages. They postulate in a subsequent research that these individuals might open up a new VL transmission channel in Spain, since domestic dogs are the disease's natural hosts.

There is certainly a need to reevaluate some components of the VL control approach if asymptomatically infected individuals might potentially serve as a source of transmission. In order to give the epidemiological and clinical information that might more extensively guide VL control strategies and promote the development of innovative diagnostic tools, more research in this field is thus necessary.

PKDL Diagnosis: An Unsolved Mysteries

Up to 50% of those who have recovered from VL may have a chronic variant of the disease termed "post-kala-azar dermal leishmaniasis," or PKDL, which develops within weeks to months after treatment. This condition is most frequent in Sudan and to a lesser degree Ethiopia. 10% of VL patients in the ISC went on to develop PKDL six months to four years later. Clinical signs include diffuse infiltration, papules, nodules, or plaques, which are hypopigmented and may be mistaken for various skin conditions. These patients do not have any physical impairments other from aesthetic disfigurement. Although PKDL mortality is minimal, PKDL patients are a largely untapped source of infection that keeps anthroponotic L alive. Illness Donovani in India. Although L. is the cause of PKDL in both Sudan and India. Donovani notes that Indian PKDL takes longer to self-heal than Sudanese PKDL (84% in 1 year).

The resources available to diagnose PKDL are insufficient. Without any parasitological proof, PKDL is diagnosed in endemic locations using clinical signs and symptoms, a history of kala-azar, and positive antibody tests. This strategy may not be accurate enough, nevertheless, since 10% of patients with PKDL had no prior history of VL and positive serological test results even years after therapy. The sole confirming test is skin slit smear microscopy, however it is difficult to perform on retinal lesions and is very painful.

Due to contamination, LST have a poor sensitivity and are challenging to cultivate parasites. Nested PCR is very sensitive, and more recent kDNA-based quantitative PCR research has shown its effectiveness in the diagnosis of PKDL. These molecular diagnostics need a highly developed laboratory and are quite expensive. There is a severe therapy gap for PKDL, and Ambisome therapies have negative effects. Rapid, non-invasive point-of-care diagnostic assays are thus urgently needed since localized VL epidemics have been connected to PKDL index cases.

HIV-VL Coinfection Diagnosed: Time for Concerted Action

How to detect and treat people who have HIV-VL coinfection is complicated by the rise of HIV and its link to VL. Due to issues with detection, reporting, and diagnosis, the true number of HIV-VL coinfection patients in India is likely undercounted. A focused reemergence of VL has been related to an index case of HIV, and patients with HIV-VL coinfection constitute an important but generally ignored reservoir of parasites (reviewed in).

By Deniau et al. and Cota et al. , numerous diagnostic techniques for HIV-VL have been evaluated for their sensitivity and specificity. Since the majority of these individuals often do not demonstrate detectable levels of antibodies, serological testing are thought to be inaccurate.

Although direct detection of parasite or a component of it in blood by PCR or qPCR is increasingly used not only for diagnosis but also for the follow-up of patients during and after treatment (reviewed in), these tests are frequently difficult to obtain in underdeveloped healthcare environments. Parasitemia is more common in HIV coinfection. Currently, guidelines on serological or molecular diagnosis of HIV-VL are not backed by conclusive data. Therefore, invasive spleen or bone marrow aspiration or a combination of RDTs employed in a diagnostic algorithm are often used for diagnosis.

CONCLUSION

The most crucial phase in VL control is to eliminate the last instance by using sensible tactics. As the arsenal of antileishmanial medications is limited and usually linked to side outcomes, this can only be accomplished with the availability of swift and affordable diagnostic tests in disease endemic locations to allow the doctors to make precise treatment choices. The term "diagnosis" has to be used extensively since it covers a wide variety of topics including infection, illness, severity, and treatment response. The traditional confirmatory diagnostic test for VL still involves the visualization of amastigote using microscopy, although this is not feasible in endemic locations. Several less invasive serological tests, such DAT, rK39 ELISA, and molecular testing, like PCR, are gaining popularity at the moment, however they are now restricted to the lab. K39 dipstick or ICT seems to be the top option for decentralized diagnosis of VL with a high sensitivity and specificity, notwithstanding the early heterogeneity reported across various manufacturers and nations. It must be used together with a defined clinical case definition owing to certain restrictions, however. At this time, there are no diagnostics that can identify asymptomatic L. forecast the development of an infection into a clinical case of VL illness. In the next years, diagnosing HIV-VL coinfection will be another issue. Last but not least, there is a definite need to close the gap between existing clinical treatment of VL practices and accessible technologies and research initiatives for VL diagnosis.

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CHAPTER 4

A COMPREHENSIVE REVIEW ON ECTOPHOSPHATASES' POTENTIAL ROLES IN HOST-PARASITE INTERACTIONS

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ABSTRACT:

Important processes for the development of infection include the interaction and survival of pathogens in hostile settings and in conflict with host immune responses. Ectophosphatases are enzymes found at the plasma membrane of cells, and instead of having their active sites facing the cytoplasm, they have them towards the external media. These enzymes can hydrolyze phosphorylated substrates in the extracellular environment once they are activated. Numerous investigations have shown that a wide range of harmful species, including bacteria, protozoa, and fungi, include surface-located ecto-phosphatases. The function of ecto-phosphatases in host-pathogen interactions is poorly understood. This article gives a summary of current research on the pathogenicity that these surface molecules cause in protozoa and fungus.

KEYWORDS:

Cell, Cytoplasm, Enzymes, Plasma Membrane.

INTRODUCTION

In all biological systems, cells are subjected to a variety of external stimuli during the course of their life cycles. Protein phosphorylation and dephosphorylation are key processes in a cell's ability to recognize internal and external signals and respond appropriately. Protein phosphatases catalyze the removal of phosphate groups from particular protein residues (i.e., dephosphorylate), while protein kinases transfer a phosphate group from ATP to a protein (i.e., phosphorylate).Numerous cellular processes, including metabolic pathways, cell-cell communication, proliferation, and gene transcription, depend on the harmony between the opposing actions of protein kinases and phosphatases. The kinome and phosphatome of a few trypanosomatids were able to be put together thanks to the entire genome sequencing of diverse bacteria. These approaches have opened up new avenues for study in the fields of biochemistry, physiology, and genetics by revealing information on the life cycles of microorganisms and by forecasting diagnostic biomarkers, cutting-edge therapeutic targets, and potential candidates for parasite-specific vaccines [1], [2].

In order to cling to and penetrate mammalian cells, parasites use a variety of surface and secreted chemicals. Certain signaling pathways, which are essential for parasite entrance and survival, are triggered by certain of these chemicals in the parasite and the host cell. Enzymes with their active sites directed away from the cytoplasm and toward the extracellular media are found in the plasma membranes of cells and are crucial for host-parasite interactions. Additional requirements for an ectoenzyme include the following: (1) the enzyme must act on an extracellular substrate; (2) cellular integrity must be preserved during enzyme activity; (3) the products must be released extracellularly; (4) the enzyme must not be released to the extracellular environment; and (5) the enzyme activity must be modifiable by nonpenetrating

reagents. This hypothesis is supported by the observation that certain bacteria include surface-located phosphatases known as ecto or extracytoplasmic phosphatases. The physiological functions of these enzymes in these cells are not yet completely understood. The most frequent phosphorylation sites in eukaryotes are found on the residues serine, threonine, and tyrosine. Phosphoprotein phosphatases (PPPs), metallo-dependent protein phosphatases (PPMs), aspartate-based phosphatases with a DxDxT/V motif (members of these three groups are Ser/Thr specific phosphatases), and the unique group of protein tyrosine phosphatases (PTPs) were thus classified into these proteins based on catalytic signature motifs and substrate preferences. Protein tyrosine phosphatases (PTPs), Cdc25, and low molecular weight phosphatases (LMW-PTPs), which share a similar motif (CX5R) in their catalytic sites, are three evolutionarily unrelated types of protein tyrosine phosphatases. Depending on whether transmembrane domains are present or absent, the classical PTPs are divided into groups of phosphatases that are receptor- or nonreceptor-type [3], [4].

DISCUSSION

These enzymes have also been categorized with the use of inhibitors, divalent cations, metal chelators, and various pH ranges. The pH range in which phosphatases are active determines whether they are acidic or alkaline. The pH range where acid ectophosphatases function best is between 4.5 and 5.5, whereas the pH range where alkaline ectophosphatases function best is between 8.0 and 9.0. The inhibitors that are traditionally used are phosphotyrosine phosphatase inhibitors ammonium molybdate and sodium orthovanadate; acid phosphatase inhibitor sodium fluoride (NaF); secreted phosphatase inhibitor sodium tartrate; alkaline phosphatase inhibitor levamisole; and phosphoserine/threonine phosphatase inhibitors okadaic acid and microcystin-LR. Ectophosphatases have been suggested to have a number of biological functions. By hydrolyzing phosphomonoester metabolites, these enzymes may give microorganisms a source of inorganic phosphate , shield them from the macrophage's respiratory burst , aid in cell differentiation , aid in the infection of host cells , and shield the cells from acidic conditions by buffering the periplasmic space with phosphate released from polyphosphates . Alkyl and aryl phosphates, as well as the phosphotyrosine analog p-nitrophenylphosphate, are examples of low molecular weight nonproteic phosphoesters that certain protein phosphatases have been reported as being active towards. Ectophosphatases are a crucial tool for pathogen survival in hostile environments and evading host immune responses because of their surface accessibility and the protein phosphorylated on serine/threonine/tyrosine residues at the cell surface. In this study, we discuss how ectophosphatase activities, especially those seen in parasitic fungi and protozoa, affect host-parasite relationships.

Ectophosphatase Activity in Infection by Protozoa

Despite the initial evidence of this activity in Trypanosoma brucei and T. brucei, little is still known about the physiological relevance of protein phosphatase activity in trypanosomatids. In 1972, there was a cruzi. The complicated life cycles of kinetoplastid parasites and the difficulty of cultivating certain of their life forms may make it challenging to investigate ectophosphatases. In their life cycles, pathogenic trypanosomatids have at least two separate host environments an insect vector and a mammal. Additionally, the capacity of each trypanosomatid genus to survive and procreate in such hosts varies. As an example, Leishmania spp. are parasites that live within cells and target macrophages for invasion. As opposed to that, T. numerous cell types, such as macrophages, fibroblasts, and myocytes are invaded and infected by cruzi. T. brucei is a mammalian host bloodstream parasite that is just an extracellular parasite. These parasites' life cycles occur in a variety of settings, necessitating frequent and significant adaptive alterations in several cell processes that alter

gene expression, protein levels, and protein modifications. In addition to these, cell surface elements are crucial for protozoan parasite survival in adverse conditions and in the face of host immune responses. Since these flagellates' phosphatase makeup is different compared to human phosphatases, the PTP family is significantly reduced while the STP family is dramatically enhanced. Since these enzymes are not very comparable to their vertebrate counterparts, they may provide good candidates for the creation of powerful inhibitors that have little impact on the physiology of mammalian hosts [5], [6].

Ectophosphatases are crucial in the interaction of cells with their environment under these circumstances, particularly as their catalytic sites are exposed to the extracellular milieu. Some protozoa parasites, including T, have been shown to include ecto-phosphatases. rhodesiense, T. congolense, T. Brucei and T. T. cruzi. rangeli, a few Leishmania species, Herptomonas muscarum, and other Phytomonas species., Entamoeba histolytica, Giardia lamblia, and Trichomonas vaginalis, among others. These ectoenzymes are sometimes referred to as membrane-bound acid phosphatases since, in general, it is claimed that their activity are at their peak in the acidic pH range. The low optimal pH and surface location of these enzymes in trypanosomatids point to their potential involvement in lysosomal digestion and/or an acidic milieu, presumably reflecting the parasite's adaptation to the intracellular or phagosomal environment.

An acidic phosphatase was cloned and purified in T. Brucei hypothesize that these enzymes might be a distinct family of ectophosphatases that do not exhibit similarity to other recognized phosphatases. These proteins seem to be involved in the control of T. Since these acidic phosphatases are produced in bloodstream forms but not in the insect procyclic form, brucei growth is impeded. Likewise, complete procyclic and circulatory forms of T. have ectophosphatase activity on their surface. Fernandes et al.'s demonstration of brucei is found in. These enzymes exhibit various behaviors, including as susceptibility to inhibitors and interference from metals. In a similar manner, L also produced and purified an ectophosphatase. mexicana, while in wild-type promastigotes it was situated in the endosomal/lysosomal compartment between the flagellar pocket and the nucleus, and the overexpression of this protein results in its extensive exposure on the cell surface. The bloodstream version of T's membrane-bound acid phosphatase showed the same results. brucei, where the enzyme is considered to help in insect development and the maintenance of endocytosis/exocytosis. The abundance of acid phosphatases on the cell may be a physiological adaptation that allows the parasite to survive in the host.

Ectophosphatase activities were discovered on the cell surfaces of all T in this circumstance. Epimastigote trypomastigote, and amastigote forms are cruzi development stages. These enzymes seem to be magnesium-dependent in amastigote forms and are capable of hydrolyzing phosphoaminoacids and phosphoproteins in physiological settings. Once T. is present, this activity could make it easier for parasites and host cells to communicate. Cruzi phosphatases cause the dephosphorylation of proteins necessary for this protozoan parasite's signal transduction pathway or cycle control. This is supported by the fact that the Colombiana strain shows Mg2+-independent ectophosphatase activity whereas the Y strain exhibits Mg2+-dependent activity. Members of these two categories vary in their behavioral patterns when taking into account their propensity to infect mammalian host cells, among other things.

The Colombiana strain's parasites looked to be more infectious to myoblasts than the Y strain's, whereas the latter is more infectious to macrophages than the Colombiana strain's parasites. The phospholipid mediator platelet-activating factor (PAF), which is interestingly connected to cellular differentiation in T. cruzi causes these parasites to secrete an

ectophosphatase, which is linked to the parasite's infectiousness [7]–[9].Protein tyrosine phosphatase inhibitor sodium orthovanadate is added to the interaction medium by L. Leishmania may cause tyrosine phosphorylation because of the large increases in parasite binding and internalization caused by amazonensis and macrophages. Under these circumstances, pathways associated to protein tyrosine kinase control Leishmania promastigote invasion, which increases ectophosphatase activity. Among these cells, amazonensis binds ligands that promote intracellular survival. Leishmania seems to reduce MAP kinase signaling and the production of c-FOS and iNOS in macrophages during macrophage infection, which increases the activity of the cell's phosphotyrosine phosphatase. These results point to a strategy Leishmania species may exploit to deactivate macrophages. And maybe by other intracellular infections as a means of communication and survival inside their hosts. In L. Additionally, donovani tyrosine phosphatase activity was found, pointing to the possibility that tyrosine phosphorylation occurs, but not via receptor tyrosine kinase or tyrosine kinase-like activities, but rather most likely as a result of the activity of atypical and/or dual specific kinases.

Furthermore, L describes a membrane-bound PTP. Promastigotes generate a large metacyclic promastigote that is transported to the cytoplasm. Although the molecule was present at higher levels in metacyclic promastigotes than in procyclic forms, the specific activity of the enzyme was reduced in the latter. It's interesting to note that L. possesses a protein tyrosine phosphatase. amastigotesdevelop to survive in mice due to a significant (LmPTP1). Although the biological purpose of this is unknown, it may play a significant role in virulence, which allows an invading disease to persist in a host. Isolated ecto-phosphatase from L. Donovani promastigotes prevent human neutrophils intact from producing superoxide anions. We may infer that parasites with higher ectophosphatase activity would be more resistant to oxidative bursts from the host's immune system, and this activity may help the parasite survive within the host.

Even though two acid phosphatases have been identified in these parasites a membrane-bound acid phosphatase (MAP) and a phosphatase that is secreted to the culture medium (SAP), as well as to the cell interface in amoebic liver abscess the function of ectophosphatases in invasive amoebiasis is still unknown. Since the invasive E. coli bacteria, these enzymes may be connected to cellular attachment mechanisms. Comparing histolytica to its noninvasive counterpart and the free-living E, the latter demonstrated much increased ectophosphatase activity.

Activities of Ectophosphatase in Fungal Infection

The tight but dynamic structure of the fungal cell wall is crucial for many biological activities, including cell shape determination, morphogenesis, reproduction, cell-cell and cell-matrix interactions, as well as osmotic and physical protection. Distinct enzymatic activity, heat-shock proteins, glycosphingolipids (GSL), melanin, histone, and integrin-like proteins are just a few examples of the many distinct cell wall components that have been studied. These elements have undergone extensive research as potential pharmacological and therapeutic targets. Although the functions of ectophosphatases in fungi are still largely unknown, their cellular distribution and their capacity to disrupt physiological processes by removing the phosphate groups from regulatory proteins point to a role for these molecules during host cell infection. The presence of surface-located acid phosphatases, called ecto or extracytoplasmic phosphatases has been demonstrated in nonpathogenic yeast Saccharomyces cerevisiae and in pathogenic species such as Candida albicans , Candida parapsilosis , Sporothrix schenckii , Aspergillus fumigatus , Fonsecaea pedrosoi , Cryptococcus neoformans and Pseudallescheria boydii [10], [11].

Additionally, the majority of the phosphatases produced under Pi-limiting circumstances are either found in the extracellular media, linked to the plasma membrane, or found on the cell wall. This theory was supported by the findings of Kneipp et al., who showed that conidial forms of F. The ectophosphatase activity of pedrosoi is controlled by exogenous phosphate. F. seems to be. pedrosoi, conidial cells produced in a Pi-depleted media demonstrated a considerably greater ectophosphatase activity than fungus cells maintained in a complete medium. In comparison to fungi producing low levels of enzyme activity, these cells expressing strong phosphatase activity were substantially better able to adhere to epithelial cells and fibroblasts . The loss of phosphate groups from host cell surface proteins was subsequently suggested to cause conformational changes and a lessened electrostatic attraction between fungal and epithelial cells. Thus, it is likely that the elimination of inorganic phosphate may open up new locations on the host surface where pathogenic pathogens can interact. Ectophosphatases seem to include sticky domains that could actively encourage the attachment of fungal cells to their hosts, acting similarly to the well-studied microbial adhesins. The functional activation of surface adhesins, which are the primary structures facilitating fungal adhesion, may be regulated by them. It's interesting to note that PAF and propanolol, which are known to activate signaling pathways and cell differentiation, supported the augmentation of F. pedrosoi ectophosphatase activity, which implies that F. One surface marker for morphological change and infection is pedrosoi ectophosphatase.

In the mold C. neoformans, a thick capsule made up of neutral and negatively and positively charged polysaccharides, is influenced by a variety of environmental factors, including the locations where the fungus infects the host. It would seem that the chemicals covering the cell wall's outer layer may be important when inadequately encapsulated cells interact with their host tissues. The capsule polysaccharides of C may conceal the accessibility of ectoenzymes to extracellular receptors. neoformans, reducing their capacity to behave as surface chemicals that affect how fungal cells interact with host cells. In actuality, several C isolates. Neoformans have activity for ectophosphatase. Although there was no association found between enzyme activity and capsular size or serotype, the levels of enzyme activity varied widely across the isolates.

Evidence suggests that the ectophosphatase activity of isolates with the same serotype of capsular polysaccharides differed significantly. Furthermore, the strain that produces a small capsule removed phosphate groups significantly less effectively than the strain that expresses a big capsule, suggesting that the capsule's presence reduces the enzyme's ability to function in this process. However, some encapsulated strains had ectophosphatase activity levels that were greater than those seen in the acapsular mutant. Additionally, it was shown that certain strains had extremely comparable levels of enzyme activity but vastly different capsule sizes [12]–[14].

Together, our findings suggest that variations in enzyme activity should result from the normal variance of ectophosphatase expression in various C. strains of Noeformans. Kiffer-Moreira et al.’s investigation of three distinct C isolates supported the earlier results. parapsilosis, including two newly identified strains (RFO and H297) as well as a strain developed in a lab (CCT 3834). They discovered that the RFO strain, which is followed by the isolates H297 and CCT 3834, had the greatest levels of enzyme activity and adherence to CHO cells. When yeasts were pretreated with sodium orthovanadate, an irreversible inhibitor, their capacity to adhere to epithelial cells was significantly diminished. Although ATPases involved in cation transport can be inhibited by sodium orthovanadate its main biological activity in living cells occurs on the cell surface because the oxidation-reduction reactions that happen in the cytoplasm lessen its inhibitory effect. Ectophosphatase from C.

parapsilosis may be regarded as a crucial virulence component. Likewise, C. Children who are HIV-positive (HIV+) for albicans have much greater ectophosphatase activity than HIV-negative (HIV) kids. The C.

CONCLUSION

The balance of serine, threonine, and tyrosine residue phosphorylation and dephosphorylation regulates signaling pathways that are crucial for influencing the outcome of several cellular operations. The extracellular medium contains phosphorylated substrates that may be hydrolyzed by ecto-phosphatases, an enzyme. To clarify the functions of ectophosphatases in host-pathogen interactions and any potential relationships between the expression of these enzymes and the clinical presentation of the illnesses, further research is necessary. Higher adhesion indices were seen in albicans yeasts from HIV+ patients, which may indicate that the activity of fungal acidic surface phosphatases plays a role in the early processes necessary for disease development. Since ectophosphatases are associated with cell development, host cell-pathogen interactions, and the outer layer, it seems sense to assume that they serve as virulence markers.

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CHAPTER 5

UNDERSTANDING HOST-PATHOGEN INTERACTIONS IN CHRONIC CNS INFECTION: TOXOPLASMA ON THE BRAIN

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ABSTRACT:

More than one-third of people on the planet are chronically infected with Toxoplasma gondii, a common obligate intracellular parasite. The capacity of a parasite to produce persistent, non-immunogenic bradyzoite cysts, which often develop in the brain and muscle cells of infected animals, including humans, is crucial to the parasite's ubiquity. Chronic infection has more recently been related to behavioral abnormalities, while acute clinical infection often entails neurological and/or visual impairment. A delicate balance between host immunity and parasite immune evasion is required for the establishment and maintenance of chronic infection. Here, we evaluate the documented effects of Toxoplasma gondii on behavior and neurological disorders and detail the known cellular interactions between Toxoplasma gondii and cells of the central nervous system. Finally, we highlight recent technological developments that will help us better comprehend host-pathogen interactions.

KEYWORDS:

Infection, Parasite, Pathogen, Toxoplasma.

INTRODUCTION

The phylum Apicomplexa, which includes internal parasites with a distinctively polarized cell shape and a complex cytoskeletal and organellar organization at their apical end, includes Toxoplasma gondii. Almost every nucleated human or avian cell may get infected by and harbor this obligate intracellular parasite. It is thought that T. principal means of transmission. Consuming rare or uncooked meat may cause gondii in humans. T transmission that is vertical is also possible. Gondii is also conceivable, and it develops when a pregnant woman has a primary infection that might cause fetal morbidity such hydrocephaly. In fact, T. Infection with Gondii is the main contributor to fetal abnormalities in the US. Depending on feeding habits and exposure to cats, who act as the disease's primary hosts and pass on environmentally robust oocysts in their feces, up to 80% of a population may be afflicted? Oocysts may contaminate food or water sources and spread disease to other warm-blooded animals for up to a year in the environment. According to a recent research, oocyst-acquired illnesses are the most clinically severe kind of infection. These infections may also be brought on by contaminated municipal drinking water in addition to direct cat fecal contact [1], [2].

The quickly replicating tachyzoite stage and the slower growing, cyst-forming bradyzoite stage are two crucial intracellular phases in the pathogenesis and transmission of Toxoplasma gondii. Latent infections in humans were formerly thought to be mostly asymptomatic. Toxoplasma, on the other hand, sprang to prominence as a significant opportunistic pathogen during the original AIDS pandemic. Through an unidentified mechanism, parasite tissue cysts burst and release bradyzoites when the host's adaptive immune response deteriorates. There is

severe morbidity, including Toxoplasma encephalitis, as a result of these recrudescent infections, which allow the parasite to transition to the rapidly-diverging tachyzoite stage.

Prior until recently, T. Despite brain alterations, gondii persistent infections were thought to be mainly benign in the otherwise healthy patient. However, more recent research using model animals has shown that post-infection behavioral alterations are evident. Additionally, links between parasite infection and neurological conditions including schizophrenia have recently been found. Therefore, it is crucial to do more research on the connections between infection and illness as well as between host and parasite. The host immunological response, which is just now being defined and understood, is crucial to these problems.

Quick Infection and Transmission

Consuming tissue cysts of Toxoplasma gondii is the most likely main infection trigger. The parasite excysts after surviving the stomach processes in order to pass into the intestinal epithelium and continue its spread. The parasite is generally protected against soluble, humoral, or cellular antimicrobial agents because of its favorable intracellular position, albeit the extent of protection may vary depending on the genotype of the parasite. As recently reviewed in, an immune response is still elicited during this acute period. Despite an inflow of lymphocytes and innate immune system cells, the parasite has evolved modifications that enable it to control the innate immune system, typically resulting in continuous growth in the gut tissue. Contrary to popular belief, it is thought that these cells, in particular dendritic cells and macrophages, are intracellularly infected and enable the parasite to spread hematogenously by acting as a "Trojan horse" [3], [4].

Once in circulation, parasites have the ability to move between infected cells while continuing to exist in the tachyzoite form before the adaptive immune response is triggered. After that, parasites find a way to restrict themselves to muscle and brain tissue. The parasites are thought to cross the blood brain barrier's endothelial cells via an unidentified technique. Recently, Lachenmaier et al. Al hypothesizes that infected mouse brain endothelial cells facilitate the migration of infected leukocytes through the blood–brain barrier. It is yet unclear whether alternative methods, such extracellular parasite barrier penetration, are used to enter the CNS.

DISCUSSION

Formation of Bradyzoite

The parasite's ability to spread through carnivory depends on its persistent, strong bradyzoite stage, which also accounts for the parasite's widespread presence. A cyst wall formed during differentiation encloses host cells that may contain 100 or more individual parasites in tissue cysts. Depending on the kind of infected cell, the change from the acute to the chronic stage is assumed to be caused by external stresses to the parasite, host, or both. Neurons and muscle cells are terminally differentiated and removed from the cell cycle, claim Blader and Saeij. They have proposed a model in which tachyzoite growth is encouraged inside developing cells, but bradyzoite development starts when tachyzoites are unable to control the host cell cycle. Although there are variants to this technique, raising the pH of the culture fluid to 8.0–8.2 is the physiologically most successful way to induce the bradyzoite stage in vitro. Prior to host cell invasion, Toxoplasma gondii exposure to an alkaline medium improves bradyzoite differentiation.

An induction approach that is less harmful to host cells is heat shock of the host cells for 2 hours prior to invasion, followed by parasite invasion for 2 hours at 37°C and subsequent

heat shock of infected cells for 12-48 hours following infection. The use of trisubstituted pyrrole, sodium nitroprusside, or sodium arsenite are all efficient chemical induction techniques. Lack of nutrients, such as the amino acid arginine, promotes differentiation and delays development. Slow development and differentiation to bradyzoites are also brought about by concurrent suppression of pyrimidine de novo production and salvage pathways. Tachyzoite replication has been found to be slowed by altered host cell gene expression, which may lead to induction of bradyzoite-specific gene expression. Thus, it seems that the parasite regularly enters the bradyzoite state in vitro when external stress is applied to it [5]–[7].

The bradyzoite stage has been the subject of several investigations because to its clinical significance and the ease with which it may be produced in vitro. The T. The gondii cyst wall membrane, which is mostly made up of glycoproteins, is considered to be essential for preserving the parasite's nutritional and structural requirements while reducing host immune system detection. Subcellular organelles also undergo additional modifications that may be seen, such as a shift away from dense granules toward micronemes and big amylopectin granules. The parasite inhibits cell division and shifts into a quiescent G0 state, and parasite eIF2 phosphorylation causes a significant slowdown in overall protein translation.It's interesting to note that bradyzoite production was increased in vitro when a parasite's abundant protease inhibitor was knocked off. At eupathdb.org, you may access the transcriptional profiles of high-resolution timecourse studies with differentiating tachyzoites. Measurements of parasite transcripts from several strains treated to various induction circumstances, such as CO2 starvation, sodium nitroprusside, alkaline medium, or Compound 1 treatment, are included in this research. These investigations' findings show a new collection of early upregulated transcripts in addition to confirming the upregulation of well-known bradyzoite markers. The bradyzoite cyst form, according to Sullivan et al., significantly contributes to Toxoplasma's success in the following ways: the cyst survives gastrointestinal processes, allowing invasion of the small intestine; the cyst is resistant to the host immune response the parasites persist without disrupting host cells throughout the host's lifetime; bradyzoites in tissue cysts are infectious, lending to carn.

Immune Reaction to Infection of the CNS

The parasite creates a careful equilibrium of low metabolic and proliferative activity after invading the central nervous system tissues while avoiding a strong host immune system activation. The host benefits when the potential for extensive immunopathology is balanced against the pathogen's rapid reproduction. Although the majority of Toxoplasma subclinical infections show this equilibrium, it should be highlighted that the interaction between different host and parasite genotypes allows for significant variance in the apparent immune response and duration of infection. The majority of information on the immune response in T. rex was published because to the challenges in investigating human CNS illnesses. Murine models serve as the genesis of gondii CNS infection. Cross-species studies of effector molecules may be challenging since there are immunological differences between humans and mice that are well documented. However, these models have greatly advanced our knowledge of how Toxoplasma infection affects cellular immunoregulation.

Tachyzoite parasites seem to infect astrocytes, neurons, and microglial cells upon entrance to the CNS, presumably with distinct affinities. Following parasite invasion, CD4+ and CD8+ T cells inflow in a mechanism that is currently poorly understood but is essential for controlling T. gondii CNS infection and that may be stimulated by the CD28 or ICOS pathways. According to two-photon imaging studies, infection and subsequent lymphocyte infiltration are known to result in structural changes to CNS tissues. Although their function is less

obvious, cellular elements of the innate response including macrophages and NK cells are also able to infiltrate the CNS during infection. The generation of IFN-gamma, which has been shown to be crucial for the inhibition of parasite reactivation in an immune cell-mediated way, is a key characteristic of influxed activated T cells. IFN-gamma is produced by microglial and other cells after infection, together with a number of other pro- and anti-inflammatory cytokines and chemokines. Since neurons constitute the predominant kind of chronically infected cell, it is possible that astrocytes and microglial cells' ability to limit parasite multiplication upon activation explains why this is the case. Additionally, host cell autophagy seems to be necessary for the parasite clearance process. Microglial cells, however, may serve as a "Trojan horse" in the spread of recurrent parasite infection, according to a recent study [8], [9].

In the course of and after an acute T. gondii, the host must maintain a balance between restricting parasite growth and guarding against harm brought on by immunity. While the immunological hyperactivity that results from subsequent exposure to T cannot be prevented, immunopathology during initial infection may be prevented by IL-10's suppressive impact. gondii, nor necessary to elicit a memory reaction. In the context of toxoplasmosis, IL-27 has also been characterized as immunosuppressive and has the potential to trigger the production of IL-10. Inducible TIMP-1, an inhibitor of matrix metalloproteinases generated by astrocytes and other microglial cells, is also thought to play a role in the local regulation of immune-related disease. MMP-8 and MMP-10, proteins involved in tissue remodeling, cell migration, and inflammation, have been shown to be expressed more often by T cells moving into the CNS after parasite infection. TIMP-1, an MMP inhibitor, prevented parasite growth by around a factor of four, although it is anticipated that unchecked MMP activity will result in more CNS damage.

The parasite is most often discovered in the CNS in the bradyzoite stage if a persistent infection has been established. Cysts were discovered throughout the brain by microscopic investigations, however they were more prevalent in the cerebral cortex, hippocampus, basal ganglia, and amygdala. First, the acute immune response may effectively eliminate cells infected with the tachyzoite stage of the infection, leaving only bradyzoite-containing cells to remain alive. This might explain why the cyst stage predominates. Second, the acute response-related increase of interferon-gamma may sustain parasite differentiation. Recent research suggests that internal cyst formations are an efficient method of immune evasion because, unlike external parasites, cyst-bearing cells are not detectable to CD8+ T cells. Alternately, the comparatively modest MHC class I expression seen in neurons may explain these findings. Furthermore, it has been shown that the presence of antigen in the CNS affects T cell behavior.

It is important to note that different changes in the host immune response have been proven to enable the illness to return, which is characterized by the parasite turning back into tachyzoites and eventually developing into toxoplasmic encephalitis. When the AIDS pandemic first started, it became clear how clinically relevant this result was. However, in the majority of immunocompetent situations, parasite infections will last throughout the whole of the host's life in a chronic subclinical form. Uncertainty exists about the frequency with which bradyzoite cysts rupture in immunocompetent hosts and swiftly reinvade neighboring cells. A powerful memory response that kills some or all extracellular parasites before reinvasion may be triggered by rare cyst discharge. Or maybe the bradyzoite cysts just have the capacity to outlive the host. It is most likely a result of a combination of these occurrences that the parasite and host maintain a stable equilibrium, making it one of the most common parasitic illnesses worldwide.

Investigating Toxoplasma gondii's Effects on Behavior

To improve their transmission, several parasites have been documented to change specific host behaviors. Toxoplasma gondii latent infection is among the most common infections in humans, although despite early research indicating harmful memory effects on mouse models, it has generally been believed to be asymptomatic. Recently, it has been discovered that the parasite may alter host behavior. Rats with the parasite were shown to have reduced fear of cats, the infection's primary host, as compared to uninfected controls, giving the parasite a sexual advantage. Researchers have begun to wonder if the parasite may have comparable effects on people as a result. It is unclear whether these behavioral alterations in the host are the result of the parasite alone or of the host's immunological reaction to the parasite. As an alternative, these impacts may be unintended consequences of the host's disease or even a fortunate result, such as motivating the host to take bigger risks in order to fulfill increased energy needs. Infected rats, for instance, are more active than their uninfected counterparts. It's interesting to note that infected rats react less neophobically to each new stimulus than do uninfected rats. While a percentage of infected rats had a possibly sexual attraction to cat-treated regions, other infected rats displayed a severe aversion to cat-odored places [10], [11].

According to the behavioral manipulation theory, parasites will selectively alter host behaviors that are crucial to their own success. However, since the brain circuits involved in anxiety, intrinsic fear, and acquired fear overlap so much, the parasite may affect all of them inadvertently. According to one study, the medial and basolateral amygdala had roughly twice as many cysts as other brain regions such the hippocampus, olfactory bulbs, and prefrontal cortex. Processing of memories and emotional responses, such as fear, is mostly carried out by the amygdala. This might be the cause of the infected mice's nonwildtype affinity to cat odor and/or their altered reactions to fear or sexual desire. Therefore, in this situation, the behavioral manipulation hypothesis would be in favor of the parasite's ability to reduce natural feline fear and maybe replace it with a new or feline attraction, while seemingly leaving other domains untouched. However, no mechanism connecting diseased areas with behavioral alterations is currently understood.

Nonmemory-related cognitive functions, anxiety, and social behavior in infected mice are comparable to controls to the extent that these are measurable; nonetheless, they exhibit substantial and extensive brain disease, motor coordination, and sensory abnormalities. Hyperactive MMP proteolysis and/or the development of new brain structures, as was previously mentioned, may both play a role in these modifications. It has been suggested that T should modify the CNS. Human hosts may also experience behavioral effects from gondii infection. According to a review in, there have been recorded links between latent Toxoplasma infections and changes in human behavior, including slower reflexes, less rule awareness, less novelty seeking behavior and more jealousy in males, and promiscuity and more conscientiousness in women. Dopamine levels in rats may be raised by Toxoplasma gondii this may be because the parasite directly produces dopamine or because it causes an inflammatory response that releases more dopamine by boosting cytokines like interleukin-2. Numerous neurobehavioral symptoms that are thought to be brought on by toxoplasmosis are correlated with dopamine's overall role in the human brain.

Psychiatric sequelae associated with toxoplasmosis

Schizophrenia is thought to arise as a result of a dopamine imbalance between the mesolimbic and mesocortical brain areas. This could make a connection between toxoplasmosis and schizophrenia possible. One of the most common and serious mental

disorders is schizophrenia. Schizophrenia is characterized by impairment in mental processing, perception, cognition, mood, and psychomotor activity, with onset often in early adulthood. The connection between parasites and the development of mental diseases, as well as personality changes and risk-taking behavior, is gaining attention. Notably, the inhibitory effect of medicines on T may enhance their antipsychotic and mood-stabilizing effects, which are used to treat schizophrenia and other psychiatric diseases. gondii in infected people . The antipsychotic haloperidol and the mood stabilizer valproic acid are two examples of this, since they most efficiently prevent Toxoplasma development in vitro but not in vivo.

Although a causal connection has not yet been shown, there is a wealth of correlating evidence. For instance, 185 Turkish non-intoxicated drivers of cars involved in crashes over the course of a six-month period had their toxoplasmosis status checked. Drivers who were engaged in collisions had a much higher likelihood of having T. Comparing the seropositive rates for gondii infection to the control group, 33% vs 8.6% were found. Seropositivity to Toxoplasma gondii has been evaluated in a variety of investigations of people with schizophrenia and other serious mental diseases, with varying degrees of correlating evidence. Additionally, the symptoms of Toxoplasma gondii encephalitis may resemble those of schizophrenia and other mental illnesses. Patients with AIDS and toxoplasmic encephalitis have experienced a large number of instances with symptoms such delusions, thinking disorder, and auditory hallucinations.

Obsessive-compulsive disorder in people has also been linked to toxoplasma gondii infection. Men were "more expedient, suspicious and jealous" as well as having "lower superego strengths and higher vigilance"; these traits are linked to drug misuse, anxiety, and personality problems. Women exhibited almost the exact opposite behavior: stronger superego strength and signs of warmth, conscientiousness, and moral commitment. However, it was shown that compared to uninfected controls, both men and women displayed more anxiety. Flegr asserts that variations in testosterone levels might also account for the observed variances. The observed behavioral alterations may be the consequence of the parasite boosting testosterone availability in order to further weaken the host's cellular defense. High testosterone people may be more vulnerable to Toxoplasma infection due to a less strong immune response. In a tiny research, it was shown that seropositive males had greater testosterone levels than uninfected men. It is uncertain, however, whether high testosterone predisposes people to infection behaviorally or physiologically, or if the parasite indirectly affects testosterone levels. The in vitro growth rate of Toxoplasma was significantly boosted in a high-throughput cell-based screening research by overexpressing 17-hydroxylase in human cells, but intracellular growth was inhibited by silencing this gene. A crucial metabolic enzyme called 17-hydroxylase is in charge of transforming compounds that resemble cholesterol into androgen precursors like testosterone. This study raises the possibility that sterols that resemble testosterone may directly promote parasite proliferation.

CONCLUSION

Due to the increasing likelihood that T. Since chronic Toxoplasma gondii infection is incurable, there may be a revived interest in antiparasitic medications. However, since medications must pass both the blood-brain barrier and the parasite cyst wall, agent development may be challenging. Additionally, even if the parasites could be eliminated from the neurons without causing more tissue damage, preexisting tissue disease could make it impossible to resolve any behavior-related consequences. A recent research discovered numerous substances that may stop T. Along with P. falciparum tachyzoites in vitro, gondii tachyzoites are also being studied for their potential antibradyzoite characteristics.

Additionally, a potential vaccine may be developed with the help of our improving knowledge of the intricate immunoregulatory mechanisms that surround parasite infection. However, it highlights the dearth of knowledge about the interaction between the immune system and the bradyzoite stage, which may be an important area for further research. Future research may focus on defining the procedure by which parasites cross the blood–brain barrier and getting a better grasp of the molecular processes by which T cells regulate infection.

Similar to how electron microscopy contributed to our understanding of apicomplexan organisms, advanced imaging techniques like bioluminescence and two-photon imaging promise to give us more in-depth, real-time knowledge about how this parasite functions and interacts with the host. Tetramer-based molecular methods also promise to provide comprehensive details on the specific roles played by antigens and host immune cells. Last but not least, host alteration by siRNA and host gene overexpression may reveal vital cellular components needed for the parasite's life cycle. Hi-throughput cell-based screening promises to significantly speed up this knowledge.

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CHAPTER 6

HOST IMMUNE RESPONSES, NEUROVIRULENCE, AND ANTIGENICITY WERE PRODUCED BY THE JAPANESE ENCEPHALITIS VIRUS

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ABSTRACT:

Infected bodily cells begin secreting various cytokines and stimulate the innate immune system in response to a JE virus onslaught. By invading nerve cells and infecting the central nervous system, viruses begin neuronal invasion. It produces neurotrophic benefits and prevents the body's natural immunity from being exposed. The virus creates an encephalitis illness that has a relatively high mortality rate in youngsters and produces acute CNS vulnerability. JEV prevents the development and spread of NCPs in survivors and causes long-term neural problems include cognitive, motor, and behavioral deficits. Body cells, however, begin TCR-mediated interactions to identify viral antigens with class I MHC complex on particular target cells and carry out mass death of virus-infected cells by enhanced CTL activity. As a result, interactions between cells and antibodies are crucial for defense against JEV. The neurovirulence, antigenicity, and host immune responses caused by the current review article's virus are detailed in depth. An increased focus is placed on clinical treatment, diagnosis, and active vaccination with possible anti-flavivirus vaccines. Additionally, worldwide eradication methods are required to eradicate JEV and improve the reliability and efficacy of immunization programs in endemic locations.

KEYWORDS:

Cells, Japanese encephalitis virus (JEV), Virus.

INTRODUCTION

The Japanese encephalitis virus (JEV) is a single stranded RNA virus that is enveloped positive and a member of the family Flaviviridae, genus Flavivirus. One of the most significant endemic illnesses in Eastern and Southeast Asia, encompassing the countries of India, Nepal, Japan, China, Korea, Thailand, Indonesia, Malaysia, Vietnam, Taiwan, and the Philippines, is JE. Recently, illness has been detected in New Zealand and continental Australia. JE is a significant public health issue that has a significant negative impact on children's morbidity and death rates. It is brought on by an awful mosquito-borne virus (arbovirus) that infects humans and is spread by Culex tritaeniorhynchus and Culex vishnui mosquitoes in rural Asia. Water birds and Culex mosquitoes, especially Culex tritaeniorhynchus, are engaged in the natural cycle of the Japanese encephalitis (JE) virus in endemic places. Pigs also play a role as an amplifying host and serve as a link to people due to their closeness to houses. These are crucial in the epidemiology, amplification, and dispersion of JEV. JEV transmission is seasonal, and it rises along with culicine mosquito populations when they engage in more frequent mass nesting in rice fields during the rainy season.

It is aided by favourable environmental factors including temperature increase, high humidity, and rainfall filling of water reservoirs, primarily in paddy-growing agricultural fields and places. Due to the fact that rural areas account for the majority of JE cases, the illness is largely seen as a rural issue. More than 70% of individuals there had experienced arbovirus infection at some stage. JEV is a reemerging virus that is thought to produce 50,000 cases worldwide each year, 15,000 of which result in death, and up to 50% of survivors are left with serious persistent neurological sequelae [1], [2].

Neurovirulence and the Flavivirus Genome

The positive single-stranded RNA genome of JEV is around 11 kb long. The virus genome only has one open reading frame, and its genes are ordered in the following order: 5′ C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-2K-NS4B-NS5 3′. It produces viral proteins that are mostly a precursor polyprotein with three structural proteins (C, prM, and E) and seven nonstructural proteins (NSI, NS2, NS2B, NS3, NS4A, NS4B, and NS5). With 619 amino acid residues and enzymatic activity including serine protease, helicase, and nucleoside triphosphatase, NS3 is a multifunctional nonstructural protein. The processing of the viral precursor polyprotein and the replication of viral genomic RNA both depend on NS3. Infected cells' NS3 protein, which is linked to microtubules and the tumor susceptibility gene 101 protein, is crucial for viral packing and the intracellular movement of different viral components. It was found in the cytoplasm of pyramidal neurons in the cerebrum, granule cells, tiny cells, and Purkinje cells in the cerebellum 12 hours after infection in the brain of a patient who had contracted JEV. One of the target cells for JEV infection is the cerebellum's Purkinje cell. NS3 is a crucial agent that causes people to become neurovirulent.

Another non-specific protein is NS1, a soluble cell surface protein that, upon peripheral viral injection, induces neurovirulence in host brain cells. It is a multifunctional protein that helps the virus invade neurons, is crucial for pathogenesis and cellular proliferation, and determines virulence. Because of this, it is utilized to produce antibodies that are protective against flavivirus. It exhibits host immunological response and also induces protective immunity in mice. It exhibits similarity with the dengue virus protein, and mouse CNS is affected by its deformation [3], [4].

DISCUSSION

Another significant envelope protein, called E, is essential for viral attachment, membrane fusion, and penetration into the host cell. It is a significant structural protein with a large number of neutralizing epitopes that facilitate viral attachment to host cells. Additionally, the E protein has a potential receptor-binding domain that triggers the host immunological response or one putative N linked glycoylation site at NS154. E proteins are a well-known target of the defending antibody response against flavivirus infection and include virulence determinants, much as NS1 viral proteins. In addition, cDNA analysis was used to identify two more structural proteins, C and prM, in the JE virus. These have glycosylation sites with potential for protection. Additionally, the prM protein in JE viruses has two potential N-linked glycosylation sites, one at the N15E protein and the other at N154. It exhibits similarities to the prM or E protein's N linked glycosylation site seen on TBEV and WNV. A deletion at the aforementioned region results in less viral release. Similar to Pv, changes in the coat and envelope proteins of HRV2 (Human rhinovirus type 2) have a meaningful role in determining neurovirulence in mice.

Chimeric viruses were created and used to demonstrate the point. Similar to this, the poliovirus exhibits PV tropism and neurovirulence in a subset of neuronal cells found in the human central nervous system. However, changes made in the premembrane (prM) and

envelope protein (E) of the Japanese encephalitis virus have shown greater protective potential. It is undeniably true that prM or E glycosylation mutants in mice improve the immunological response. Additionally, the N-linked glycans of viral proteins are crucial in controlling the immunological response in host cells. In particular, neutralization epitopes that may change a protein's susceptibility to proteolysis are crucial for maintaining proper antigenic conformations. Even while several biological neurovirulence indicators have been found to promote neurovirulence the molecular basis of virus-specific components that contribute to virulence is still unclear. Because they were encoded in many locations of neurotropic RNA viruses, they could be extremely selective and more conclusive than biological markers. The development of neurovirulence is caused by mutations in structural and nonstructural viral proteins. These might be deletion- or reversion-type items. The flavivirus genome, which encodes the hinge region of the envelope protein, included single site alterations that significantly increased the pathogenicity of the virus in mice and monkeys.

However, changes made to the vesicular stomatitis virus's matrix (M) protein result in neurovirulence. These VSV M protein mutations may serve as vaccine delivery systems. In contrast, the measles virus has low neurovirulence in humans but exhibits substantial neuroinvasivness in animal models. Similar to how the poliovirus generates a high level of CNS sensitivity and develops in neural cells, the host only exhibits a low level of neurovirulence. After intracerebral inoculation of experimental mice, further serial passages of the yellow fever virus (YF 17D) in the mouse brain increase neurovirulence and decrease survival time. Increased neurovirulence in the severity of infection may be the result of viral invasion in nerve cells, primarily in the brain. Additionally, it exhibits a virus that changes its course of propagation primarily in the neuroaxis, brain, and spinal cord. More remarkably, it has been discovered that the JRES and internal ribosome entry site include nearby stem loop structures that work together to define neuro-pathogenicity. It is impossible to rule out the possibility of more scenarios. For instance, fewer connections between viral antigens and host immune cells increase the likelihood that viruses would invade neurons more often, which might result in greater pathogenicity. In such a situation, cellular and humoral defenses cannot effectively combat the virus, the chemicals it produces, or even the body’s own infected cells [5]–[7].

When a virus infects a healthy person, it spreads to numerous glands where it replicates. The incubation period is between five and fifteen days, after which it reaches the circulation. In the end, the virus finds a home in the brain, where it begins to cultivate and cause major issues. When the JE virus is inoculated into a person, it immediately begins to replicate in Langerhans dendritic skin cells and moves to draining lymph nodes. Viral infection, the disjunctive ability of bodily cells, and other variables all play a significant role in the formation of an early immune response at this period. Viralemia starts to spread as soon as the infection touches secondary lymphoid tissue, including the liver, kidney, and spleen. The virus escapes from blood, bypasses the immune system, and travels through a hematogenous pathway to the brain and spinal cord. Infected inflammatory cells or active replication in epithelial cells are other ways in which it might breach the blood brain barrier.

In addition, the meninges, the tissue that covers the brain and spinal cord, swells and gets contaminated. At this point, the neck of the JEV-infected patient becomes stiff and uncomfortable. The patient has fever, a strong headache, nausea, and vomiting by days two or three. Other significant signs and symptoms include brain swelling, loss of balance and coordination, paralysis of specific muscle groups, tremors, seizures, and lapses in consciousness. These signs and symptoms are often seen in patients. Due to severe

dehydration, the patient's face takes on the look of a hard mask and loses weight. Patients' symptoms worsen as their excessively high fevers persist. Additionally, the patient's body temperature is increasing and they are experiencing brain malfunction, which puts them in a coma and causes them to pass away within 7 to 14 days. Patients who made a full recovery had persistent neurological problems such deafness, mental retardation, emotional instability, and hemiparesis in addition to irreversible neuronal impairments brought on by brain trauma. The interaction of virus attachment proteins (VAP) with cellular receptors is necessary for the onset of viral infection. It is well established that the interaction between VAP and its cellular receptors affects host range, tissue tropism, and viral pathogenesis. Once the characteristics and function of the virus receptor are known, this might eventually result in the development of powerful antiviral drugs. On the host cells, however, there are no known cellular receptors for JEV.

Pathogenesis

The JE virus quickly grows in nerve cells, multiplies wildly, tests the body's defenses, and causes serious pathological alterations in people. Before crossing the blood-brain barrier, it escapes from the bloodstream and spreads to neuronal cells through a hematogenous pathway, replicates peripherally and briefly causes viremia, or it may pass via the cerebrovascular endothelium or the olfactory nerves. Virus avoids exposure of body’s natural defenses, imposes neuropathogenesis, and creates neurotrophic effects that culminate in encephalitis syndrome or acute sensitivity to CNS. It primarily targets NCPs (neural progenitor cell) pools, and it begins the invasion and death of immune cells via cytolytic processes.

It also limits the development and multiplication of these cells by interrupting the cell cycle progression. Additionally, it attacks cerebellar Purkinje cells, which results in neurological symptoms such ataxia. IFN-, which facilitates noncytolytic virus clearance, is produced by T cells and activated uninfected brain macrophages when nerve cells are particularly infected. Additionally, the virus secretes proteins and factors, disrupts the balance of cytokines, and silences MHC I that is found on the membrane surface [8], [9].

The JE virus invades healthy body cells, triggers immune reactions, and leads to a number of pathological alterations in the host. It kills big neuronal cells, stimulates microglia, and produces a powerful inflammatory response. The central nervous system's (CNS) resident immune cells, or microglial cells, play a crucial part in the host's defense against invasive microbes. These cells release cytokines when activated, including tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1), which may have harmful effects on the brain. These cells also release other soluble components that intensify disease, including neurotoxins, excitatory neurotransmitters, prostaglandins, reactive oxygen and nitrogen species, and neurotoxins.

Therefore, the pathophysiology of flavivirus encephalitis seems to be a mix of directly virally caused cell damage and the host inflammatory response, which results in a significant reduction in the number of NCPs cells and severe neuronal destruction in patients owing to neuronal apoptosis. The body's inherent immunity is not exposed at this stage as a result of the virus's bloodstream escape. As with CMV, which targets and infects embryonic NPCs, it causes severe neuropathogenesis and induces neurotropism. It also suppresses the proliferation and differentiation of these cells. It has an immediate impact on memory and learning, and it causes long-term cognitive defects/disorders such motor and behavioral abnormalities (neurological sequelae) in newborns or surviving young children. HIV infection produced similar invasion NCPs that caused these cells to become dormant.

JEV-Related Innate Immune Responses

Body cells are able to run a general defensive or innate immune responses in response to JEV infection. However, after interacting with virus-infected body cells, many cytokines of type 1 interferons, such as TNF and interferon, are stimulated and begin to be produced. These cytokines work in concert to have powerful effects on the cells and organs and to inhibit virus replication by inducing an inflammatory response. Additionally, IFN- and IFN-binding to NK cells causes lytic activity, which kills virally infected cells. Furthermore, IL-2, a cytokine that is generated very early in response to viral infection, significantly increases the antiviral activity of NK cells. IFN- stimulates macrophage activation, increasing class II MHC molecule expression, cytokine production, and microbicidal activity. Additionally, it controls innate immunity and offers nearby cells antiviral defense. Additionally, ICAM1, ELMF, and class I MHC molecules are produced as a result of cytokines, which also aid in reducing infection. Thus, innate immunity seems to fight against JEV to some degree, as shown by an enhanced level of alpha interferon, although therapy with interferon alpha did not benefit the patients. Similar to this, sialokinin-1, a salivary protein that is released from the salivary glands of Aedes aegypti or Culex pipiens mosquitoes, functions as an immunomodulatory factor that up regulates the TH2 cytokines, IL-4 and IL-10 and down regulates the production of IFN- . It speeds up the multiplication and spread of viruses.

Additionally, big granular lymphocytes or polymorphonuclear cells phagocytose viruses and virus-infected cells in the bloodstream and within other organs to destroy them. Virus-induced lymphokines and cytokines activate these cells, and the complement system, a multicomponent enzyme cascade, attracts them to the site of infection. In addition to this, neutrophils, macrophages, and monocytes choose to employ chemical antimicrobial secretions for both oxidative and nonoxidative assault and attempt to create a multifaceted, highly effective coordinated defense. However, microbicidal actions, both oxygen-dependent and oxygen-independent, also assist in viral eradication. Meanwhile, the NADPH phagosome oxidase enzyme complex (Phox), which aids in the mass eradication of viruses, produces both reactive oxygen species (ROS) and reactive nitrogen species. Additionally, neutrophils carry a variety of weapons as they go from the circulation to the infection site. These cells have a number of toll-like receptors on their surfaces, which enable them to sense viruses directly, adhere to them, and exhibit phototropism, as well as mark them with antibodies, complement, or both. Acute phase proteins like C-reactive proteins, which aid in the anti-inflammatory response to viral infection, are also used in other defensive processes. Numerous innate defense mechanisms thus work to stop the virus invasion, but rapid virus population growth and recurrent exposure to viral proteins aggravate pathogenesis, rob cells of their immune systems, and cause sudden drops in soluble factors [10], [11].

JEV-Related Adaptive Immune Responses

The resulting adaptive immune responses are much targeted; they exhibit antigenic variety, specificity, immunologic memory, and self/non-self-identification. Antibodies first identify antigenic epitopes, and then immune cell surface receptors and secreted signal molecules cross-check viral proteins. Additionally, the immune system produces a variety of viral antigen recognition molecules in order to establish crucial molecular contacts. As a result, immune cells can distinguish between two protein molecules released by two distinct viruses simultaneously. In adaptive immunity, lymphocytes and antigen-presenting cells collaborate. Normal B-lymphocytes that were antigen-loaded, however, transformed into effector plasma cells. Through repeated cell divisions and the production of hundreds of antibody molecules per second, they multiplied clonally. Major effector molecules of humoral immunity are secreted antibodies.

However, these antibodies work with complement proteins to destroy viruses by lysing the virus directly or by opsonizing the virus and removing it from the body via phagocytic cells. However, JEV chooses to use tactics to avoid destruction caused by complement and secretes proteins that attach to the 4b complement component and block the traditional complement pathway. The JE virus therefore continuously modifies its antigens, particularly epitopes, or creates antigenic variants to change itself into new regionally adapted strains that may be better capable of causing high intensity infection. As a result, while simultaneously trying to avoid the host body's defenses, the virus alters itself and advances via major mutations made in the neurovirulent determinant proteins or its domain. Additionally, newly developed/emerging virus strains induce more deadly encephalitis outbreaks in the absence of protective immunization. Thus, humoral immune responses are crucial for preventing JEV infection in both human and nonprimate species. However, when an antibody-operated defense fails quickly, humoral immune responses are similarly compromised, which contributes to the early mortality of JEV-infected patients. Additionally, cellular immunity serves as the first line of defense against JEV by identifying infectious pathogens via cellular receptors on T cells, including CD8 and CD4. Nevertheless, in response to a JEV infection, both innate and adaptive immune responses are triggered, and it has been shown that these immune reactions lower serum viraemia and viral loads in infected tissues.

Additionally, interactions between microbial elements and receptors on macrophages result in soluble proteins that promote adaptive immune responses, which aid in the removal of viruses. A TH cell becomes activated, undergoes metabolic change, and starts to secrete different cytokines when it detects and interacts with an antigen-MHC class II molecular complex. In order to initiate the manufacture of new components or to go through differentiation, the cytokines (soluble proteins) interact with receptors on distinct cell types. Finally, signal exchange occurs between soluble molecules and receptor molecules that are attached to cell membranes, or between membrane-bound compounds that are present on two distinct cell types. Similar to how antigen-presenting cells work with T cells to trigger humoral and cell-mediated immunity in patients.

JEV Immune Response Mediated by Cells

Cell-mediated immunity is crucial in the widespread destruction of virally infected cells. For the eradication and control of viruses, it is crucial. It is coordinated by the TH or TC cells' release of IFN-. With the aid of IL-2, an uninfected or naïve T cell (CTL), which is incapable of killing viruses when exposed to their released antigens, became infected and produced CTLs. Now, it produces significant cytotoxicity against the virus and expresses a variety of effector molecules on it. These effector CTLs are able to identify particular target cells that have antigen class I MHC complexes and eliminate a significant percentage of virus-infected cells after they have multiplied. In contrast, following exposure to flavivirus-infected cells, flavivirus antigens limit CTL release and inflammatory cytokines. Additionally, by producing broad immunological suppression of B and T cells or macrophages, antigenic differences among flaviviruses elude the immune response. By using cytolytic processes, it causes immune cells to be destroyed, altering their function, creating an imbalance of cytokines, or suppressing MHC I on the membrane surface. At the same time, viral proteins may attach to MHC class I molecules in the endoplasmic reticulum and stop them from moving to the cell membrane. Another hypothesis is that the viral proteins might prevent protein kinase C from transmitting signals to T cells during activation. That is why the virus is able to successfully lyse a variety of cells owing to coordinated enzyme membrane assault. Similar to humans, spider monkeys, who are typically resistant to JEV-induced illness but have demonstrated sensitivity to other viruses, had their T-cell activity suppressed. CTLs and effector molecules

destroy virus-infected cells as a result of the cell-mediated immune response, while NK cells and macrophages kill viral cells via antibody-dependent cell mediated cytotoxicity (ADCC).

Additionally, in T-cell deficient mice, passive transfer of immunological spleen T cells might result in permanent cell-mediated immunity. T lymphocytes are thought to be necessary in these animal models for recovery and defense against a deadly challenge caused by JEV. When thymus-depleted mice were exposed to JEV, they had a reduced anti-JEV antibody response, although T-cell responses are crucial for producing B-cell immunity after a JEV infection. As an alternative, JEV primed pure T cells given intracerebrally (i.c.) together with 10x LD50 JEV have shown protection. Depletion of either population eliminated the protective capacity of transplanted effectors, suggesting that a population of CD4+ and CD8+ T cells inside T cells is required for functional protection against JEV. It demonstrates that, despite CD8+ T cells, only CD4+ are necessary for protection. But other researchers rejected it, claiming that neither enhanced JEV primed T cells nor B cells were discovered to be able to protect mice from JEV infection. However, in the case of other flaviviruses like dengue virus, CD8+ T-cell activation may help to reduce disease pathogenesis or immunological pathology. Furthermore, immunization with the envelope DNA vaccine in knockout mice revealed that the protection was solely lost in the Ig and CD4+ cells [12], [13].

Dendritic cells and macrophages are two distinct immune system antigen-presenting cells that often play a dual function in human JEV infection. In addition, activated TH cells release a variety of cytokines, including as IL2, IFN-, TNF, and IL-6 which may modify the cellular function of the JE virus and protect the host against viral infection. By producing CD8 Tc cells and CD4, TH1 cells, which begin to function as an antiviral defense, these effector chemicals aid in the establishment of a cell-mediated immune response. Additionally, it causes an increase in the production of all of these cytokines in the spleen as well as an increase in the levels of IL-4 and TNF in the serum of JEV-challenged mice. As a result, both TNF and IFN may contribute to the quick removal of viruses from the peripheral nervous system (CNS) and may also aggravate inflammation there. Among these, IFN- produces an antiviral state in cells whereas IL-2 indirectly encourages the development of a larger population of effector T-cells from CTL precursors. Natural killer cells (NK cells) are activated by these in conjunction with IL-2 and IFN- and are capable of destroying vast numbers of virions as well as one's own virus-infected cells . Additionally, throughout the viral life cycle, double-stranded dsRNA molecules produce IFN- and IFN-, which aid in the eradication of virus-infected cells. Similar to this, CTLs use the E protein of JE and nonstructural proteins produced by uninfected cells as their targets for death. Additionally, complement activated cytotoxic lymphocytes can destroy vast numbers of viruses and virus-infected cells with the aid of antibody molecules, acting as a second line of defense.

Dendritic cells and macrophages are two distinct immune system antigen-presenting cells that often play a dual function in human JEV infection. Following viral infection, these cells become active and begin to produce a variety of cytokines, which may modify how the JE virus functions in cells. However, TNF, IL-12, and IL-6 production as well as the elevation of CD80, CD86, and CD40 costimulatory molecules as well as chemokine receptor alterations are all linked to DC maturation and enhanced antigen presentation. Additionally, the toll-like receptor (TLR) molecules found on cell surfaces aid in the formation of innate immune responses and the recognition of pathogens. As a consequence, JEV causes functional impairment of DCs, CD4+ and CD8+ T cells, which causes patients to develop significant immunological pathogenic alterations that culminate in severe CNS illnesses. Therefore, JEV infection of macrophages led to an increase in cytokine and costimulatory marker production that stimulates cellular community.

JEV-Induced Humoral Immune Response

Humans and non-primate individuals are mostly protected against JEV by humoral immune responses. It begins following detection of virus by T helper cells, which swiftly react to viral antigens. TH cells exhibit and deliver viral antigens to B cells together with macrophage. After 7-8 days of viral infection, these antigen-loaded B cells transformed into plasma cells, which begin secreting antibodies. As a result, an antibody-mediated humoral immune response is triggered, which attempts to suppress the infection by attaching to a molecule on the host cell membrane. By attaching to epitopes required to promote the fusion of the viral envelope with the plasma membrane, antibody molecules prevent viral penetration. If the induced antibody is complement activated or bound, both of these processes may agglutinate viral particles and serve as an opsonizing agent to aid in the phagocytosis of the viral particles by Fc or C3 b receptors. Neutralizing antibodies from JEV-infected cells, however, prevent the virus from replicating and lessen the cytotoxic effects it causes. 5-7 days after infection, the host's immune system is triggered by the structural and non-structural proteins produced by the JE virus via its interactions with the host's cells.

Specifically in responder cells, flavivirus infection evades immune response. It is the major cause of the possibility that different individuals' immunological responses to JEV infection may vary. Although there is a chance that immune responses will stop the spread of the virus in its early stages, the presence of inhibitory substances generated by the virus causes a rapid breakdown of the antibody-operated defense, which destroys the humoral immune response and kills JEV-infected people. Within 5-7 days, patients' cerebrospinal fluid (CSF) naturally secretes significant quantities of IgM molecules, which are either favorable prognostic signs or markers of JEV-generated infection. Additionally, it increased in the CSF and serum of the majority of symptomatic individuals. Its peak concentration was measured starting on day 9 after the beginning of clinical illness that may be identified using an anti-JEV IgM capture ELISA.

CONCLUSION

A range of chemicals are used by viruses to trigger inhibitory reactions in order to infect their human hosts. However, a variety of innate immune responses work in response to a virus assault to stop the virus invasion, but rapid virus population growth and frequent exposure to viral proteins worsen pathogenesis. Additionally, a sequestered defense created by active body cells and chemicals prevents viral identification and eradication. As a result of immunological suppression and a rapid drop in soluble components, there is an increase in virus-infected cells. Acute phase proteins like C-reactive proteins, which aid in the anti-inflammatory response to viral infection, are also used in other defensive processes. What's more intriguing, however, is how viruses defend themselves against cells, chemicals, and anti-infective drugs. In order to understand the natural history of the virus and its infectivity in various hosts, particularly in various animal models, research of flavivirus evasion is crucial. JEV is an RNA virus with higher potential for viral gene integration into the host genome. It will increase a virus' ability to infect the host, and the virus will be better able to counteract the effects of innate and humoral immune reactions. Additionally, local genotype changes that resulted from reversions, deletions or additions, or positional shifts of sequences in the viral genome have the potential to generate new JEV strains. It could produce highly contagious gene products or proteins that broaden the range of disease occurrences, accelerate neuropathogenesis, and enhance lethality. It's interesting that other highly dangerous long-term viruses are also present in the same environment, which makes it easier to spread and may have a different potential for infection from host to host.

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CHAPTER 7

THE CONFLICT BETWEEN DENGUE VIRUS INFECTION AND HOST IMMUNE RESPONSES AND ITS EFFECT ON THE PATHOGENESIS OF DENGUE DISEASE

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ABSTRACT:

The single-stranded RNA virus known as dengue virus (DENV) belongs to the genus Flavivirus and is spread by mosquitoes. The illness is prevalent to tropical and subtropical regions of the globe and is categorized as dengue fever, dengue hemorrhagic fever, and dengue shock syndrome depending on symptoms and severity (mild to severe). Among the several human cell types that DENV targets, innate immune cells including monocytes, macrophages, and dendritic cells are capable of initiating quick inflammatory responses. These cells are also important antigen-presenting cells that turn on the long-term memory-activating adaptive immunity. The present state of knowledge about the interconnected elements of DENV structure, viral infectivity, cellular receptors, innate immune response, and adaptive immunity is summarized in this work.

KEYWORDS:

Dengue, Disease, Host Immune, Infection, Virus.

INTRODUCTION

Single-stranded RNA virus of the genus Flavivirus, the dengue virus (DENV), is transmitted through arthropods. DENV-1, -2, -3, and -4 are its four closely related but antigenically different serotypes. More than 100 tropical and subtropical nations throughout the globe have an endemic virus. There are currently no particular treatments or vaccinations available to treat illnesses or stop the spread of DENV. Undifferentiated fever, dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) are all conditions brought on by DENV infection. The WHO described DF as an acute febrile illness with two or more signs of headache, retroorbital pain, myalgia, arthralgia, rash, and other symptoms in their record from 1997. DF symptoms might linger for 2–7 days. The following characteristics are used to identify DHF: a prolonged high fever, a propensity to bleed, hemoconcentration (>20%), and platelet counts (100,000). In addition, DHF is divided into 4 classes based on the degree of bleeding and plasma leakage. DHF grades III and IV are mentioned in DSS. Rarely do instances of severe dengue disease meet all four of the requirements to be classified as DHF patients in many places. As a result, WHO established revised categorization criteria for the severity of dengue illness in 2009; nonetheless, the 1997 case definition is still in use. Children are the most susceptible at the moment, accounting for 2.5 to 40% of all hospitalized dengue cases; DHF and DSS [1], [2].

The following 3 parameters, virus strain virulence, host genetics, and host immunological state, are the main causes of DHF in addition to diet, age, and sex. Usually, 5-7 days following a blood meal from an infected mosquito, dengue infections start to manifest. Viremia in human peripheral blood reaches its peak in the first few of days of an acute

sickness before rapidly declining. Higher viremia titers were seen in DHF patients compared to DF cases. Immune responses are generally necessary for the clearance of DENV infection; however, since DHF is linked to secondary infections and manifests symptoms as viremia declines, DHF/DSS is believed to be an immunopathological outcome. DENV infection is allowed in the three different kinds of human cells: monocytes, macrophages (M), and dendritic cells (DCs). These cells are important innate immune system phagocytic cells that find and eliminate invading infections. Additionally, they are antigen-presenting cells important for the beginning, development, and polarization of adaptive cellular immunity. Immune regulation may be significantly impacted if DENV targets these cells. In order to better understand the pathophysiology of DHF, we compiled current research on viral architecture, host cells and receptors, and host innate and adaptive immune responses in this study.

Dengue Virus Life Cycle and Structure

A lipid bilayer from the host cell covers the DENV particle. The DENV genome is an 11 kb single positive-sense RNA molecule that codes for 10 viral proteins: 3 structural proteins (NS)—NS-1, 2A, 2B, -3, -4A, -4B, and -5—and 7 nonstructural proteins (NS). Through receptor-mediated endocytosis, DENV penetrates host cells. Major roles in viral attachment, endocytosis, uncoating, and fusion are played by the E proteins on the surface of the virus. Domain I (130–185), domain II (50–130, 185–300), and domain III (300–400) are the three functional domains found in the E protein. While domain III is thought of as a binding domain that attaches to cellular receptors and directs DENV particles to the host cell's endosomal compartments, domain II is thought to include a fusion peptide. In an acidic environment inside the cellular endosomal compartments, viral uncoating releases viral RNA into the cytoplasm. Immediately after viral uncoating, protein translation starts as a positive sense (messenger) RNA [3], [4].

The genome RNA first generates an unbroken polyprotein, which is later broken down into separate proteins in the ER lumen and cytoplasm of the host cell. PrM, E, NS1 and NS4B cleavage occurs before that of the other NS and C. For the cleavage of viral proteins, host cell proteases and viral protease NS2B-NS3 are necessary. In the so-called viral replication complexes (RCs) located close to the cellular membranes in the cell cytoplasm, the NS3 is also a helicase and collaborates with the NS5, an RNA-dependent RNA polymerase, to allow viral RNA replication. While freshly generated RNA joins with C to create a nucleocapsid at the cytosolic side of the ER membrane, newly synthesized E and PrM enter into the ER membrane. The nucleocapsids link with the membrane-bound PrM and E via a process known as "budding," creating offspring viral particles in the rough ER cisternae. These viral particles go to the Golgi apparatus and are subsequently delivered to the cell surface by secretory vesicles for extracellular release. At a late stage of viral replication, a Golgilocalized furin protease cleaves prM, and the mature progeny virion released extracellularly contains M protein.

DISCUSSION

Protein modification occurs in the ER and Golgi apparatus together with protein translation continuing in the ER of the host cell during viral replication. N-linked glycosylation, which involves attaching a high mannose core to the amide nitrogen of asparagine (Asn) in a consensus sequence of Asn-X-Ser/Thr, where X may be any amino acid, is one of the most prevalent protein modification techniques. The protein modification begins early in the ER during protein synthesis and continues when the protein is transported from the ER to the Golgi. The originally connected glycan is further altered at the Golgi via a difficult

remodeling or trimming process, resulting in different oligosaccharide forms. Glycosylation may enhance correct protein folding that is necessary for protein functioning, disrupt virus-receptor interactions, change antigenic structures identified by host immune cells and antibodies, and thus affect viral reproduction and infectivity.

N-linked glycosylation has recently been shown to influence DENV infection, genome replication, and progeny virion packaging. At Asn67 and Asn153, the DENV E protein is glycosylated. While the Asn-153 glycosylation site of E is conserved in the majority of Flaviviruses, the Asn-67 location is exclusive to DENV. Other glycosylation sites may be found in NS1 at locations 130 and 207 as well as at positions 7, 31, and 52 in PrM. Different effects of E protein glycosylation on viral infectivity and replication depend on the receptor types and host cell types examined. For mammalian cells (such as Vero cells and BHK cells), eliminating Asn-153's glycosylation by gene mutation decreases the DENV's capacity to infect host cells, but removing Asn-67 has no effect on the viral genome's replication or infectivity but increases the rate at which virus offspring are assembled. Asn-67 may thus play a crucial role in the correct folding of the recently generated E protein, virion assembly, and viral release in mammalian cells. Removing glycosylation at either Asn-153 or Asn-67 by gene mutation is not as important for mosquito cells (C6/36, a cell line derived from the larvae of Aetis albopictus) as it is for human cells. When the Asn67 or Asn67/Asn153 mutants were introduced into C6/36 mosquito cells, a compensatory mutation, K64N, was introduced, restoring glycosylation in the region.

West Nile virus (WNV) provides direct evidence of the alteration of glycan compounds on the surface of Flaviviruses that may affect viral tropism. Both the liver/lymph node-specific ICAM-3 grabbing nonintegrin (L-SIGN, also known as DC-SIGNR or CD209L) and the dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN, CD209) are expressed on various cells in various tissues and organs. While its homolog L-SIGN is expressed on liver sinusoidal endothelial cells, DC-SIGN is found on dendritic and macrophages in lymphoid and cutaneous organs. For DENV to engage with the carbohydrate recognition domain (CRD) on the DC-SIGN molecule, E protein must be glycosylated at position Asn-67. WNV does not exhibit N-linked glycosylation on Asn-67 as DENV does. WNV preferentially infects L-SIGN cells whereas DENV is equally infectious for DC-SIGN and L-SIGN cells. Glycosylation at Asn-67 eliminated this selectivity and made both DC-SIGN and L-SIGN cells equally susceptible to WNV infection. Studies have shown that whereas complex glycans, especially N-acetylglucosamine ended structures, were crucial for interaction with L-SIGN, mannose-rich glycans on WNV were not necessary for its interactions with DC-SIGN. According to this research, the location of N-linked glycosylation on the E protein molecule controls the kinds of glycans that are integrated, influencing viral tropism for cells that express either DC-SIGN or L-SIGN [5]–[7].

The routes for glycan production do not need a genetic blueprint. Glycan structures change across species and are influenced by variables that may vary substantially between different kinds of cells. These elements make it more challenging for us to comprehend the intricate nature of viral glycosylation. Glycan microarray, an emerging technique, may be an effective method for profiling glycan compounds crucial for immunological identification and infectivity, which is useful for the development of vaccines and drugs.

Identification of DENV Infection Receptors and Host Cells in Vitro

It has been shown that DENV infection may occur in vitro in a wide variety of host cells. African green monkey kidney cells (Vero cells), baby hamster kidney cells (BHK cells), mosquito cells (C6/36), and other cell types are susceptible to DENV infection. Laminin

receptor and other polypeptides that have not yet been completely described are examples of known cellular receptors on these cells. Monocytes, M, DCs, endothelial cells, and hepatocytes from humans have all been reported to be infected with DENV. Heat shock protein (Hsp)70 and Hsp90 , GRP78 , and heparin-sulfate are cellular receptors for hepatocytes. Mannose receptor (CD205), CD14-associated protein (CD14-AP), CLEC-5A (CD32, CD33), heparin-sulfate (CD29), and DC-SIGN (CD209) are examples of the cellular receptors for monocytes and M. Cellular receptors for DCs include DC-SIGN. Compared to adult DCs, immature DCs have increased DC-SIGN expression levels. Infectivity varies dramatically between immature and adult DCs. DCs are the most receptive cells for DENV infection among monocytes, M, and DCs. The greater infectivity in DCs is attributable to increased receptor-mediated absorption as well as increased genome replication and the synthesis of de novo viral proteins. Since the shortened DC-SIGN without the endocytosis domain could not prevent viral reproduction, some claim that DC-SIGN just helps to concentrate the virus on the cell surface and that the internalization of the virus relies on another protein.

Endothelial cell receptor has not yet been discovered. The liver sinusoidal endothelial cells' receptor is considered to be the DC-SIGN homology L-SIGN. Multiple proteins may be employed as receptors, as shown by the interaction of DENV-4 with two surface proteins of 40 and 45 kDa (a putative heat shock protein) in C6/36 cells and a receptor of 50 kDa with DENV-2, -3, and -4 . Heparin sulfate and two 44 and 74 kDa cell surface proteins promote DENV binding for Vero cells.

These results demonstrate that the carbohydrate residues play a critical role in viral binding to Vero cells as well as C6/36 cells. A glycosaminoglycan called heparin sulfate is found in the cell membrane of the majority of cells. Heparin sulfate is thought to concentrate viruses on cell surfaces, while DENV endocytosis may be reliant on another molecule. DENV-2 and -4 infections have been linked to heparin sulfate.

Identification of In Vivo Host Cells for DENV Infection

The histochemistry of postmortem samples from deceased dengue patients has been one method used to detect host cells in naturally infected persons. By using in situ RNA hybridization or immunofluorescent labeling (e.g., NS-3), the DENV genome and immunofluorescent staining of DENV protein antigens are mostly identified in phagocytic cells in the lymph node, spleen, and lung. Additionally, perivascular cells in the brain, hepatocytes in the liver, and endothelial cells in the spleen were shown to be infected with DENV. DENV antigens were found in CD14+ monocytes in peripheral blood. These findings revealed that the target cells for DENV infection include tissue M, blood monocytes, liver hepatocytes, and endothelial cells. It should be noted that DENV viremia is said to be negative during the period of defervescence and before to the commencement of DHF; as a result, the aforementioned histochemistry tests may more clearly depict a picture of late stage dengue tropism.

To provide some insight on a dynamic view of DENV tropism, a humanized mouse model may be helpful. This study demonstrated that DENV initially appeared (on day 1) outside the spleen's follicle-like structures, where T and B cells are housed, and subsequently on day 10 within those structures. DENVs were discovered outside the follicle regions between days 14 and 18. In the bone marrow, a similar pattern was seen. These findings showed that DENV targets DCs, M cells, and monocytes before T and B cells. These cells carry DENV to T and B during their migration, and the infection subsequently spreads to other organs including the liver and lung [8]–[10].

Use of Receptors and Virus Virulence

There is currently little knowledge about in vivo receptor use during spontaneous DENV infection and how it influences the severity of dengue sickness. Receptor preference is a significant factor in tissue tropism and virulence of the virus. A few animal studies might provide some insight into this area of study. A variant isolate of DENV-4 with the mutation Glu327-Gly in E domain III was created by adapting a DENV-4 isolate in DBS-FRhL-2 cells. In comparison to the unpassaged DENV-4, this mutant virus had greater affinity for heparin sulfate and decreased infectivity and immunogenicity in rhesus monkeys. By increasing systemic viral burdens, a mouse-passaged DENV strain with lower affinity for heparin sulfate was shown to produce severe illness in mice, according to another research. The serum of infected animals included greater amounts of TNF-, a recombinant virus with a lesser affinity for heparan sulfate, and had a longer serum half-life. It also had larger systemic viral loads. It's likely that varied affinities to heparin sulfate might direct viruses to various organs where the cell types or microenvironments that house DENV do not favor DENV reproduction or spreading in the best way.

On a genomic level, the function of DC-SIGN in DENV pathogenesis has been discovered. A single nucleotide polymorphism (SNP) analysis connected the polymorphism (336 A/G; rs4804803) in the CD209 promoter region with disease severity or protection. The research examined two genotypes of this promoter regionA/A and A/Gand discovered a high correlation between the GG/AG and DHF risk of rs4804803, whereas the AA genotype was linked to protection against DENV infection. In vitro produced DCs from the AG genotype expressed more DC-SIGN than those from the AA genotype. The fact that increased DC-SIGN expression was associated with higher cytokine output rather than infection level is perplexing. Innate immunity may have a substantial role in the severity of the illness as shown by the fact that TNF-, IP-10, and IL-12p40 were much greater in DCs from the AG genotype than the AA genotype.

Antibody-Dependent Enhancement (ADE)-mediated infection

The patient's prior DENV exposure is one of the main risk factors for DHF/DSS. The majority of primary DENV infections cause noncomplicated DF and the long-lasting establishment of both humoral and cellular immunity, which both protect the host against reinfection from the same serotype. Although this antidengue immune response has a cross-reactive character, it does not provide other serotypes with long-term cross-protection. Instead, it was shown that DHF was linked to subsequent infections, indicating the immunological underpinnings of dengue pathogenesis. According to the ADE hypothesis, later heterologous DENV infections are made more infectious by nonneutralizing serum Abs from an earlier exposure. As part of the ADE process, Ab binds to DENV to generate immune complexes (ICs), which then bind to FcR and boost the absorption of DENV by cells that have the FcR protein. In vitro studies on ADE revealed that it may be found in a number of primary cell cultures and cell lines, including mature DCs, monocytes, and human plasmacytoid DCs. All cell types deemed to be dengue target cells based on in vivo histology contain FcR, and all of them exhibit ADE-supporting properties in in vitro tests. Using monocytes as an example, DENV infection was less than 1% in the absence of immunological sera but may grow by >10-fold in the presence of sera Abs . Sera from participants in prospective cohort studies from endemic areas of the globe were tested in in vitro ADE assays to examine the function of ADE in the pathogenesis of dengue fever. The clinical severity or viral load of secondary DENV infection did not correlate with the ADE titers in preillness serum, indicating additional variables are crucial in the pathogenesis of

DENV infection. These considerations could include innate immunological reactions brought on by ICs of Ab-DENV [11], [12].

Innate Immunity Was Triggered by Immune Complexes (IC)

M removes the bulk of ICs created between Abs and pathogens from the bloodstream in the liver and spleen. The pathogens are degraded by enzymatic activities in the lysosomal compartments as a consequence of the IC being phagocytosed by binding to FcR produced by phagocytic cells. In addition, NK cells are capable of recognizing foreign antigens expressed on the cell surface (such as Ab-DENV bound to FcR on the cell surface or NS-1 expressed by infected cells), which causes the mechanism known as Ab-dependent cellular cytotoxicity (ADCC) to be triggered and kill the infected cells . Additionally, IC may cause the complement system to become active, damaging the infected cells and limiting viral spread. All of these innate immune pathways play significant roles in preventing pathogenic infection and are quickly activated upon pathogen invasion. In the interim, inflammatory cytokine/chemokine responses are linked to the death of target cells. It has been shown that ADCC has a protective effect against DENV secondary infection. Using preexisting DENV-positive plasma that was acquired prior to heterologous secondary DENV-2 and -3 infections via a prospective cohort study of Thai schoolchildren, a research assessed the degree of ADCC in a 51Cr-release assay. The key idea behind this work is that the NK cells destroy DENV-infected cells by recognizing the IC produced on the surface of infected cells (plasma Abs bind to DENV antigens released by infected cells). The findings indicated a correlation between greater plasma neutralizing Ab activities and higher ADCC activities. Although this link was absent in presecondary DENV2 infection plasma samples, higher ADCC activity in presecondary DENV3 infection plasma samples was associated with lower plasma viremia levels. The clinical outcomes of disease severity in secondary infections were not overtly correlated with ADCC activity, however the lowest ADCC activities were discovered to correspond with DHF of DENV-3 secondary infections. ADCC could aid in the early in vivo regulation of secondary DV3 viremia.

CONCLUSION

Regarding the viral and host cellular molecules involved in DENV-receptor contact and infection, significant advancements have been achieved. The dynamic process of DENV tropism, the preference of host cells and receptors involved in the main or secondary, and in the early or late phases of DENV infections, are not well known. Most critically, nothing is known about how glycosylation affects receptor binding, viral tropism, and infectivity pathogenicity. Innate immunological mechanisms including complement activation and ADCC are seldom taken into account in current investigations of Ab-mediated neutralization/enhancement. Future research should concentrate on these difficult areas. Furthermore, to better understand the protective immunity, future investigations on adaptive humoral and cellular immunity should make use of a distinct population: those who had DENV asymptomatic infections.

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CHAPTER 8

TRANSFERRIN: CELL SIGNALING AND ENDOCYTOSIS IN PARASITIC PROTOZOA

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ABSTRACT:

The most common metal in the human body is iron, which is also the fourth most prevalent element on Earth. Because virtually all creatures need iron for a variety of biological processes, this element is essential to life. This is true of pathogenic organisms, who are leading the fight for iron with the human host. Through a number of iron-containing proteins, including transferrin, the most recent controls Fe concentration. The transferrin receptor keeps iron away from infections and delivers it to any cell that need it. As a result of the development of certain transferrin receptors located on the plasma membrane and absorbed by endocytosis, parasites have evolved a number of methods to get iron. Proliferation depends on signal transduction pathways that are connected to receptor activation. Because of their selectivity or because of variations from their human counterparts, transferrin receptors and other proteins with actions in signaling networks are significant targets for therapeutic research. In this study, we characterize proteins involved in signal transduction, particularly those involving transferrin endocytosis, and we contrast these processes with those in T. Bruno, T. leishmania, E. coli, and T. cruzi parasites called histolytica.

KEYWORDS:

Eukaryotes, Parasites, Prokaryotes, Plasma Membrane.

INTRODUCTION

Prokaryotes and eukaryotes, which make up the majority of living things, use iron (Fe) as a cofactor in a wide range of proteins with critical roles. Fe is necessary for many biological functions, including gene control, oxygen transport, DNA synthesis, respiration, and the tricarboxylic acid cycle. However, this element has a considerable potential for toxicity to biological macromolecules. Therefore, precise methods to control its intake and storage are needed to maintain cellular Fe content. Fe is absorbed daily at a rate of around 1.5 mg with a typical diet. In the duodenum, proximal jejunum, and upper section of the gut, enterocytes carry out intricate procedures that allow for fe absorption. It is possible for nonhaem Fe or hem Fe to be absorbed. Several import proteins are involved in the Fe absorption pathway for the two ionic forms of iron, Fe2+ and Fe3+. A major source of Fe for omnivores is heme Fe from hemoproteins, which is easier to absorb than nonheme Fe from grains and vegetables [1], [2].

Fe may go to a variety of locations inside the enterocyte. The iron pool within the cell determines the Fe's final destination. As a result, Fe may either accumulate inside of cells or be expelled from them into the bloodstream. Ferroportin1 is a protein that has been found specifically for the export of Fe to the circulatory system. The enterocytes' basolateral membrane contains the multipass protein ferroportin1. Once Fe has been exported by ferroportin1, it must undergo transformation in a process that involves reoxidizing Fe2+ to

Fe3+ by ferroxidases such ceruloplasmin and then loading Fe3+ onto transferrin (Tf). Because cellular Fe induces ferroportin1 overexpression, which is reduced by hepcidin, these proteins are able to control iron efflux and, as a result, iron absorption. Hepcidin reduces Fe efflux by binding to ferroportin1 and causing its destruction.

The metabolism of cellular Fe is controlled by Fe. As part of posttranscriptional regulation, iron regulatory proteins 1 and 2 (IRP1 and IRP2) bind to iron responsive elements (IREs) found in the untranslated regions of the mRNAs encoding TfR1, stabilizing it and increasing the number of receptors in the membrane and Fe levels when Fe levels are low. Conversely, when Fe levels are high, ferritin synthesis rises and the receptor mRNA is destabilized, resulting in low Fe entry. Fe levels in bodily fluids are regulated in the blood by Fe bound to Tf, which is the primary protein for transporting Fe in plasma. Even though Fe is the most important nutrient for practically all species, it is not available naturally free and has poor bioavailability and solubility, thus all organisms have made tremendous attempts to get it.

Transferrin

Tf, a glycosylated Fe3+-binding protein that is present in blood plasma, lymph, and other bodily fluids and whose main job is to transfer Fe to all cells, scavenges Fe exported to the serum. Keeping free Fe at a very low concentration, about 1018 M, prevents harm from occurring and denies bacteria the Fe they need to develop. This is another job of Tf. Tf plays a significant role in the body's ability to fight against infections [3], [4].

DISCUSSION

Tf is a single 80 kDa polypeptide with two homologous N- and C-terminal lobes joined by a brief middle region. Only 30% of the protein's binding sites are typically filled by ferric Fe (Fe3+). Tf binds one Fe3+ ion in each of its two lobes; Fe is bound more firmly and released more slowly in the C-terminal lobe. Synergistic binding of a carbonate or bicarbonate anion is necessary for iron binding. A special issue of Biochimica et Biophysica Acta (BBA), general themes, with the title Transferrins:Molecular processes of iron transport and diseases 1820(3), 2012, just published an entire series of reviews on Tf. The liver, central nervous system, testes, ovaries, spleen, mammary glands, and kidneys all participate in the synthesis of serum Tf. Tf is a protein that may be found in all organisms, including bacteria, animals, and algae . Interestingly, Tf is lacking in nematodes, and regrettably, there is no proof that parasitic protozoa have this protein.

Receptors for Transferrin from Mammalian Cells

Tf receptors (TfR) are used by cells to take up Fe coupled to Tf; as a result, the biological purpose of the particular receptors is to bind Tf on the cell surface and take it up. The tyrosine kinase-linked receptor family includes TfRs, which include an inherent tyrosine kinase that participates in signaling cascades. Mammals have two TfRs: TfR1 and TfR2. Both TfRs are homodimeric transmembrane glycoproteins that are selective for Fe-loaded Tf (holoTf), with TfR1 having the higher absorption affinity for holoTf and now receiving the most research. With a small contribution to Fe absorption, TfR2 is essential for iron homeostasis and shares 45% of the amino acid sequence with TfR1. TfR1 mediates Tf internalization at concentrations of less than 0.3 mol/L, while high Tf concentrations have been associated with low affinity absorption of holoTf that is not mediated by TfR1. Although TfR2 has been suggested as a receptor involved in this low affinity uptake, more cells than only a few organs express this receptor, thus low affinity uptake is more widespread. Other proteins, such as proteoglycans through fluid phase endocytosis in hepatocytes and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) on the macrophage

cell surface, bind and absorb Tf with modest affinity. It is significant to highlight that some proteins, particularly glycolytic enzymes, have been shown in both prokaryotic and eukaryotic cells to possess multiple functions. Binding Tf to get Fe is one of these new functions.

TfR1 has a molecular mass of 190 kDa and is a homodimer connected by two disulfide bridges. The receptor is composed of three domains: extracellular, which is the largest one and has the Tf binding site, and transmembrane. Tf that has been absorbed by uptake receptors with high and low affinities is delivered to early endosomes as explained below. Tf changes shape and dimerizes upon binding to the extracellular domain of the TfR on the plasma membrane. This change enables the activation of kinase activity, and it gets phosphorylated. The endocytosis carried out by clathrin-coated pits allows the Tf-TfR complex to enter the endocytic pathway. For the separation of pits from the plasma membrane and the development of coated vesicles, dynamin's activity is essential. Ferric iron is released in 2-3 minutes when the Tf-TfR complex enters a special endosomal compartment that is acidified (pH 5.6) [5]–[7].

Through endocytic recycling chambers (ERC), Tf lacking Fe (apoTf) attached to the receptor is successively transferred to the plasma membrane. In order to bind additional Fe, the receptor dephosphorylates, releasing apoTf outside the cell. All proteins involved in signal transduction rely on ligand-induced receptor activation for function. Tf functions as a growth factor by way of the TfR. Due to its trafficking via the endosomal system, it plays a crucial role in the control of embryogenesis, cell development, motility, proliferation, differentiation, glucose metabolism, and apoptosis. Similar to mammalian cells, parasite TfR enhances Tf absorption and, as a result, Fe uptake via the production of a particular receptor or binding protein that is linked to the progression of the infection. Pathogens' TfRs play a key role in determining their virulence, and depending on the environment in which an infection occurs, the receptor may or may not be expressed. In this sense, parasites in blood vessels express TfR to bind Tf present in this environment; furthermore, some parasites express TfR inside cells, such as Leishmania, and it has been shown that this parasite developed strategies to increase the presence of Tf inside the parasitophorous vacuole where Leishmania lives. These complex Fe systems produced by TfR expression guarantee their success as parasites, host colonization, and infection establishment.

TfR in Parasitic Protozoan Organisms

The colonization of the host is essential for pathogen infection. The capacity to colonize relies on the presence of nutrients and growth factors like Fe. The interaction between parasitic organisms and their hosts is particularly complicated since the hosts need to sequester this mineral to keep it out of the infections' reach while still obtaining Fe from their food and meeting their own Fe requirements. The iron-containing protein Tf performs this function, however protozoan parasites lack the ability to produce Tf or Tf-like proteins, which would aid in their acquisition of Fe. These organisms have evolved methods to get host Fe via various and distinct routes or steal it from host Fe deposition sites since they are extremely reliant on an abundant supply of host Fe. These strategies include the release of certain Tf proteases, the presence of reductases that seize host proteins containing Fe, or the use of particular TfR or Tf binding proteins. To guarantee parasite pathogenicity and proliferation, TfR must be present and function properly.

TfRs have been identified in many Plasmodium species. , Tritrichomonas , Trypanosoma brucei , Trypanosoma cruzi , and Leishmania species. , and Entamoeba histolytica , among others. It is amazing that these parasites produce receptors with comparable functions and

recognition of the same carrier proteins as the mammalian cell, despite the fact that some of these receptors have distinct structural characteristics and others use entirely different mechanisms while having the same purpose. Utilizing certain receptors to acquire growth factors or nutrients guarantees that signal transduction, which began upon ligand binding at the plasma membrane and continues after internalization, is prolonged in time. The TfRs from parasites, mostly those with signal transduction investigations, have been included in this study and compared to what is now understood about mammalian cells.

Brucei trypanosoma. The protozoan Trypanosoma brucei is the culprit behind African trypanosomiasis. Due to the danger of infection for millions of individuals and the toxicity of the existing chemotherapies, this condition is exceedingly serious. This protozoan has two broad phases in its cell cycle, one occurring in humans and the other in the insect vector, the tsetse fly (genus Glossina). Fly intestine is the site of the first infection (procyclic stage). The parasite divides into the epimastigote stage before moving on to the metacyclic stage when the infection spreads to the salivary glands. The parasite is introduced into the mammalian host at this point. The parasite exists in its slender form inside the mammalian circulation and, when required, changes into its stumpy form to prepare for a subsequent insect infection. This parasite faces harsh Fe shortage circumstances when in the circulatory stage. Available as Tf is the only source of iron supplied by the host [8], [9].

Consequently, T's bloodstream develops. brucei expresses a Tf receptor (TbTfR) that mediates Tf endocytosis at the plasma membrane; this receptor has previously been discovered and is physically quite distinct from the host TfR. TbTfR is made up of a complex of proteins that are encoded by the ESAG6 and ESAG7 expression site-associated genes. The glycosylphosphatidylinositol (GPI) anchor of ESAG6 is responsible for securing the receptor to the plasma membrane. Both ESAG proteins must be bound together in order for Tf to bind. The gene for the variable surface glycoprotein (VSG), which makes up the parasite's outer layer, cotranscribes with ESAGs. VSGs exhibit the antigenic variation adaptation mechanism. Sustained infections may arise as a result of this procedure. The flagellar pocket is a distinctive feature seen in the majority of trypanosomatid protozoa that enables nutrition intake at a particular membrane location. The flagellum protrudes from an invagination of the cell membrane. Tf undergoes endocytosis in this structure. The internalization of Tf occurs via a different molecular mechanism than that seen in mammals.

Tf is internalized through clathrin-dependent endocytosis when it binds to the TbTfR, which is attached to the membrane by the GPI tail. The release of Fe from Tf is made possible by the endosome's low pH. Tf has a poor affinity for the TbTfR at this pH, so the T releases it and transports it to lysosomes where it will be degraded. T. brucei cathepsin B) and TbCATL (T. brucei cysteine-protease rhodesain or cathepsin L activity). To bind fresh Tf and the Fe it carries, TbTfR is recycled to the cell surface. The key distinction from mammalian cells is that the Tf is transferred to the extracellular media while still connected to the receptor in order to bind fresh Fe. Tf may, however, be broken down by parasites for nutritional reasons. The TfR plays a significant role in parasite adaptation and multi-host colonization. The parasite may utilise Tf from many sources since TbTfR has a low level of specificity for Tf, giving it the chance to expand its host population to include both people and cattle. The diversity of species that may be infected by parasite organisms depends on the utilization of Tf from various sources.

Comparable to the T. The brucei parasite is T. Cruzi infects its hosts, including humans and invertebrates, at certain times during its life cycle. The triatomine insect, which feeds on trypomastigotes found in an infected mammalian host's circulation, is the invertebrate host. The parasites travel to the posterior gut after transforming into epimastigotes in the gut of the

vector. After that, they change into infectious trypomastigotes, which the vector injects subcutaneously with infectious feces. Once inside, parasites enter a variety of cells using a lysosome-mediated mechanism, differentiating into amastigotes that multiply and transform into trypomastigotes that release parasites into the bloodstream. From there, the parasites can invade additional cells or infect vectors that feed on the host.

T. Epimastigotes and cruzi amastigotes that are developing in cell-free media need large quantities of Fe to live, yet oddly, at these stages, they can get Fe from human Tf. Specific TfR that are not present in the trypomastigote form are present in the flagellar pocket of amastigotes. Tf is absorbed by receptor-mediated endocytosis, and the 200 kDa molecular mass of TcTfR exhibits structural similarities with human TfR. However, the parasite consumes Tf at the cytostome/cytopharynx via a TfR during the epimastigote stage of the life cycle. Similar to the flagellar pocket, this structure is an invagination of the membrane, but the cytostome extends deep into the cytoplasm in the direction of the nucleus. Small, uncoated vesicles transport the Tf receptor-mediated uptake to the reservosomes. Uncoated vesicles' involvement indicates that the TfR is not recycled to the membrane [10], [11].

However, morphological investigations have shown that Tf internalization occurs via a clathrin-independent and cholesterol-dependent endocytosis route, despite the fact that this parasite possesses clathrin that might engage in endocytosis. Utilizing certain inhibitors of endocytic pathways allowed researchers to pinpoint this mechanism. Similar to T's internalization, clathrin-dependent. Brucei should not be ruled out; blockage of the cholesterol-dependent route did not affect cell proliferation, suggesting that it may be a secondary endocytic mechanism. Correct Tf internalization requires an unidentified interaction of the cytostome with the flagellar complex. Because the trypomastigote is the natural form that would encounter human Tf, it would be fascinating to know if this route of Tf entrance is constitutive or whether it relies on the stage of the parasites' life cycle.

At least 20 pathogenic obligatory intracellular species, including Leishmania major, L., are responsible for the leishmaniases and deadly visceral leishmaniases, which affect animals and have a wide range of clinical signs. Little, L. Brazilianis, L. Latina, L. L. amazoniensis. In addition, L. donovani. An estimated 150 million persons are affected globally, with 2 million new cases reported year. An infected sand fly bite (dipteran insect) introduces metacyclic promastigotes (the infectious form) into a mammalian host, where they begin the infection. The parasites are detected within parasitophorous vacuoles (PVs) after being phagocytosed by macrophages; these acidic structures resemble phagolysosomes and contain specific lysosomal enzymes. Promastigotes change into amastigotes within the PVs. The parasites multiply and cause cell lysis; the liberated parasites may either infect nearby cells or be phagocytosed by nearby macrophages. During blood meals, infected cells are consumed by sandflies, which subsequently get infected. Amastigotes change into promastigotes in the stomach before migrating to the proboscis to begin a new infection.

The production of a particular receptor would be helpful because the promastigotes may come into contact with Tf from the circulation after the parasites are discharged and before they are phagocytosed. Promastigotes were shown to have a distinct and saturable TfR that was identical to the TfR seen in mammals. The L's TfRs. and infantum (LiTfR). Major (LmTfR) were defined as a structurally distinct monomeric glycoprotein of 70 kDa that is an integral membrane protein. In both of L.'s developing types. Tf is bound by nonspecific, saturable Tf binding proteins in chagasi, promastigotes, and amastigotes. Unfortunately, no description of the Tf endocytic mechanism exploited by this parasite exists.

Typically, the mammalian host cell will internalize leishmania amastigotes, however in the case of L. Additionally, they are capable of being ingested by Amazoniensis promastigoteswhere they may subsequently live and grow within PVs. The PV's promastigotes and amastigotes have highly limited access to vital Fe, and Leishmania parasites have evolved a number of coping mechanisms for living within the mammalian host. One method involves merging the PV with several separate vacuoles and then merging the resulting vacuoles with endolysosomal compartments. Proteins peculiar to each of the vacuoles have been detected in association with PVs, which led to the discovery of this. The mammalian Tf-TfR complex, which is typically found in early and recycled endosomes, is associated with the PV in this form on the tenth day of infection. Additionally, Tf was discovered to be delivered to the PV and then endocytosed by intracellular amastigotes, suggesting that infection time may enhance the endosomal delivery to the PV. Depending on the Leishmania species, multiple processes might be used to get iron. Tf from mexicana-infected macrophages was not found in the PV, but the survival of amastigotes in this setting suggests the existence of a different iron source.

The TfR recycling control on macrophages would also be affected by leishmanial infection, causing a Tf disease where Tf might enter other late or lysosomal compartments and likely be delivered to PVs. Tf is transferred to compartments rich in cysteine proteinase after being endocytosed by Leishmania intracellular parasites. Here, this protein is broken down. Another method of getting iron from the Tf-TfR complex inside the acidic PV might behave similarly to how endosomes do, in which the Tf loses its affinity for iron but remains bound to the receptor, and iron release may be aided by Tf degradation by cysteine proteases secreted by living amastigotes or released by the lysis of dead parasites. Then, this element is transported using a parasite-associated or -secreted reductase, such as the Leishmanial Iron Transporter 1, or LIT1. LIT1 is crucial for Fe acquisition because it transforms Fe3+ into Fe2+ for transmembrane transport, enabling Fe to be internalized by the parasite. Enough Fe is provided by this iron transporter for Leishmania to develop and become virulent within cells.

Histolytic Entamoeba. E. Human parasite protozoan is called histolytica. It causes amoebiasis, a worldwide illness characterized by the development of intestinal ulcers and dysentery. The parasite has the ability to enter the liver, lungs, and brain under certain circumstances. E. Each year, histolytica infects 500 million individuals, causes illness in 50 million, and results in the deaths of 100,000 people. E. Fe is a vital need for histolytica. This need in the colon may be met by bacteria, phagocytosed red blood cells, or host endocytosis of proteins containing iron. Tf in the circulation and liver serve as the Fe source during invasive amoebiasis; the parasite would benefit by using ferritin, a Fe-storage protein, in this organ. In order to guarantee that it receives the Fe required for colonization and infection, the amoeba has evolved two distinct ways for getting Fe from Tf . When Tf concentrations are low, between 1.1 and 5.6 nM, internalization occurs by particular EhTfbps of 70, 96, and 140 kDa molecular mass, as previously demonstrated. Another method is through receptor-independent internalization, which is active at high Tf concentrations (micromolar range). Tf is absorbed by two methods with varying affinities depending on the Tf concentration, much as in mammalian cells. To ascertain the connection between the existence of a particular receptor with low or high binding affinity for Tf and Fe requirement, further research must be done.

The enzyme acetaldehyde/alcohol dehydrogenase-2 (EhADH2) was recognized as the 96 kDa EhTfbp. The other EhTfbps (70 and 140 kDa) remain unidentified. The growth and survival of E depend on this enzyme. histolytica and enables the parasite to convert acetyl-CoA into

ethanol and get energy via the fermentation of glucose. On the cell surface and in the cytoplasm, this enzyme binds proteins from the extracellular matrix. Given that it does not bind Tf in the presence of Fe (apoTf), this protein may be involved in the binding of Fe from Tf. Other glycolytic enzymes with diverse roles in this parasite have been identified, including enolase, which affects the activity of the DNA methylation-catalyzing Ehmeth enzyme.

In human and murine macrophage cell lines, surface-localized GAPDH performs a new role with TfR, as previously documented. Fe that is linked to Tf may interact with GAPDH. When combined with Tf, this enzyme is transported to the early endosomes.

Staphylococcus aureus and Staphylococcus epidermidis, two bacteria that can remove Fe from Tf via a receptor-mediated mechanism, were shown to have the same enzyme with a comparable activity. It's interesting to note that these enzymes also bind plasminogen, plasmin, lysozyme, myosin, and actin in addition to fibronectin and laminin from the extracellular matrix. Due to their capacity to perform several functions, these proteins have been referred to as moonlighting or multifunctional proteins. Hexokinase, lactate dehydrogenase, and -enolase are other glycolytic enzymes having numerous uses unrelated to glycolysis.

The protozoan parasite life cycle provides an explanation for why these organisms need a complex network of cell surface signaling molecules. For instance, E. In the intestinal milieu, histolytica must contend with bacteria for Fe, other nutrients, and available space, and intra- or extracellular trypanosomatids in the mammalian host must acquire Fe and other nutrients that are present in very low amounts. Additionally, in order to govern the many phases of their life cycle and elude host defenses or control their invasive behavior, these parasites need to perceive a variety of stimuli. Upon invasion, parasites are still faced with a variety of difficulties that call for their capacity to cling and collect enough nourishment. Utilizing a vast signaling network may thus be extremely helpful since the survival of these parasites within their host needs a significant capacity to recognize and adapt to environmental stressors.

CONCLUSION

Numerous diseases have shown the significance of efficient Fe absorption for virulence; yet, despite significant advancements, surprisingly little is known about the signal transduction pathways activated by Tf endocytosis in order to receive Fe. There are considerable gaps in our knowledge of these mechanisms, despite the fact that the overall picture shows commonalities with the mammalian host. Finding signaling proteins can help researchers discover novel elements crucial for parasite adaptability to the host environment. The signal transduction mechanisms in the studied organisms have been difficult to compare, but it is possible that they are very similar. As was noticed, this similarity may be preserved in both intra- and extracellular parasites, despite the fact that they respond to Fe absence in different ways. A number of proteins have changes between them and their mammalian counterparts that might be employed as therapeutic targets to cure illnesses brought on by these parasites. This has ramifications for biomedical research to create novel chemotherapeutic techniques.

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CHAPTER 9

THE MALARIA PARASITE'S SURVIVAL TECHNIQUES IN RED BLOOD CELL AND HOST CELL POLYMORPHISMS

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ABSTRACT:

The host cell is dramatically altered by parasite development within the erythrocyte, which helps the body absorb nutrients from the extracellular environment while also contributing to the symptoms of severe malaria. The interactions between the Plasmodium parasite and its metabolically severely impaired host cell, as well as the natural selection of a large number of polymorphisms in the genes producing hemoglobin and other erythrocyte proteins, are the main topics of the present research. About 200 Plasmodium species have been identified, and they cause malaria in certain monkey, rodent, avian, and reptile lineages. Although there have been sporadic reports of jumps between host species, the Plasmodium species exhibit strong host species specificity. Two such jumps are thought to be responsible for the human origin of P. vivax, when an ancestral parasite switched hosts from macaque monkeys to humans, and for P. knowlesi recently.

KEYWORDS:

Malaria, Nutrients, Plasmodium, Parasite.

INTRODUCTION

There are now five Plasmodium species known to cause human malaria. These species all go through the same basic life cycle, which alternates between the human host and the anopheline mosquito. When a female anopheline mosquito carrying Plasmodium looks for a blood meal, the cycle starts when the sporozoites are injected into the dermis. After infecting hepatocytes while migrating to the liver, sporozoites enter a clinically quiet period. Asexual sporozoite replication occurs between 5 and 16 days later, depending on the Plasmodium species, and each infected hepatocyte releases thousands of merozoites into the blood. Some sporozoites from P. vivax and P. ovale are predisposed to grow into nondividing hepatocytic forms, which may stay dormant in the liver for months to years before becoming active and causing recurrent infections.

Each merozoite enters the bloodstream, invades an erythrocyte, settles in a membrane-bound vacuole that it has created for itself, and goes through repeated cycles of growth, division, and invasion over the course of one day, two days, or three days, resulting in malaria symptoms and complications. When both of these gametocytes are ingested by the mosquitoes during blood meals, the Plasmodium life cycle continues. A fraction of developing merozoites develops into male and female gametocytes. Gametocytes undergo fertilization and maturation in the midgut of the mosquito, creating an infectious ookinete that travels through the midgut into the hemocele and matures into the oocyst in which sporozoites are produced. When the oocysts are completely developed, they rupture and release sporozoites, which go into the salivary glands of the mosquito to begin a new life cycle [1], [2].

Of the five human malaria parasite species, P. falciparum is the most lethal because host erythrocytes infected with its mature forms avoid the splenic clearance by sequestering in capillaries and microvenules of the brain and other vital organs, a process that is uncommon with erythrocytes infected by other human malaria parasite species. The malaria-associated pathology only manifests during the blood stage of infection. The parasite also exhibits a family of functionally redundant ligands that may enter human cells, and it can change its antigens to thwart host defenses, which is a major danger to the capacity to regulate infections by this lethal parasite. The dramatic host cell changes that occur as the parasite grows inside the erythrocytes make it easier for the parasite to obtain nutrients from the extracellular environment that the host cell is unable to provide, but they also have the unfavorable effect of exacerbating the symptoms of severe malaria. The interactions between the Plasmodium parasite and its metabolically highly reduced host cell, the emergence of novel physiological traits induced by the pathogen in the host cell, and finally the natural selection of a large number of polymorphisms in the genes encoding hemoglobin and other erythrocyte proteins are the main topics of the current paper.

Invasion of Red Blood Cells by Parasites

Mammalian erythrocytes, which are mainly devoid of biosynthetic pathways, metabolically slow, malnourished, and lacking in internal compartments, are infected by the malaria parasite P. falciparum. The internalization of Plasmodium merozoites into the erythrocytes occurs around 30 seconds after first contact and may be separated into two phases: preinvasive and invasive phase. During the preinvasive phase, the erythrocyte plasma membrane underwent waves of deformation that quickly stopped, leaving the merozoite connected to the erythrocyte by its front end. It is believed that a localized influx of calcium ions prompted by merozoite contact is what stimulates the host cell's membrane deformation caused by erythrocytic cytoskeletal alterations, which increases the area of contact between the merozoite and the host to aid in the merozoite's apical reorientation. As the merozoite must reorient and make contact with the RBC before invasion, the length of time and degree of deformation may depend on how near the apical end was to the erythrocyte surface on first contact [3], [4].

DISCUSSION

The RBC receptors and parasite ligands involved in this process, however, are yet unknown. G protein-coupled adrenergic receptors on the RBC membrane have been implicated in membrane deformability during merozoite invasion, according to Murphy et al. This is most likely due to cyclic AMP-induced alterations in the stiffness of the underlying cytoskeleton. Merozoite rhoptries and micronemes are thought to secrete proteins like P. falciparum reticulocyte-binding protein homolog and erythrocyte-binding antigens, which are involved in tight junction formation, during the invasive phase of the parasite life cycle. TJ is moved from the apical end to the posterior end of the merozoite in a complicated sequence of actions driven by the parasite actin-myosin motor after the formation of TJ between the parasite and RBC. The host cell then undergoes echinocytosis in response to invasion, potentially as a result of water loss from the erythrocyte induced by an outflow of potassium and chloride ions. The opening of Gardos channels in the erythrocytic membrane, which is triggered by an input of calcium ions into the erythrocyte to reseal the erythrocyte membrane behind the invading merozoite, may be the source of the potassium loss. Depending on how many times it has been infected, the iRBC takes several minutes to return to its usual shape during the postinvasive phase, which is also when merozoites change into amoeboid ring-stage.

The severe symptoms of malaria are brought on by the parasite's infection and growth within the red blood cell, and a significant portion of this intraerythrocytic development happens in the parasitophorous vacuole, a compartment that serves as a bridge between the parasite and the host cell's cytoplasm. The exclusion of important erythrocyte membrane proteins from PVM and the contents discharged of rhoptries and micronemes from the apical end of parasite during the process of erythrocyte invasion suggest that it is parasite derived, even though the molecular mechanisms causing the formation of PV remain unknown. However, the discovery of multiple erythrocyte membrane proteins in the PVM by Bietz et al. at or shortly after parasite invasion implies that its genesis is more complicated. The host cell goes through significant changes during parasite development, including the formation of nutrition transfer between the host cell cytoplasm and the parasite inside PV, across PVM, and preservation of the host cell's electrolyte balance. Additionally, the iRBC requires surface modification in order to participate in the associated processes of sequestration and antigenic variation and import nutrients not easily delivered by the RBCs.

iRBC Transporting System

The host cell is unable to meet the parasite's high nutritional needs due to its rapid growth and replication, so new permeability pathways must be created to allow the parasite to absorb various nutrients from the extracellular milieu and the cytosol of the host cell. The high amounts of hemoglobin within would typically stimulate fluid to migrate in, expand, and rupture the cell if the RBC membrane became very porous. The parasite consumes more hemoglobin than it requires for its own proteins, mostly via the cytostome, a huge phagosome-like structure seen in the intraerythrocytic trophozoite stage of the parasite. This prevents the invading RBC from bursting too soon. Although hemoglobin serves as the parasite's primary supply of amino acids, several other amino acids, such as isoleucine and pantothenic acid, must be generated from the host cell's surrounding media. For the acquisition of nutrients from the extracellular environment, three models have been proposed: the parasitophorous duct model, the metabolic window model, and the sequential uptake model. In all three models, it is suggested that solutes enter the parasite from the PV through transporters located within the parasite plasma membrane. The sequential uptake model has lately drawn a lot of interest as a working hypothesis, despite the fact that a comprehensive model describing the absorption of a range of chemicals is a significant difficulty [5]–[7].

However, due to the absence of a protein synthesis route in noninfected RBC, it has long been assumed that the proteins facilitating transport across the erythrocyte membrane are parasite generated. The erythrocyte membrane contains endogenous host cell proteins that are protease sensitive and involved in solute transport following activation by infection-induced oxidative stress. Auxiliary parasite proteins may be necessary for activation, and studies have demonstrated that these channels are anion-selective and that either the parasite exported kinases affect the activity and/or substrate specificity of otherwise quiet endogenous transport proteins. Consequently, proteins released by parasites that are involved in transporter activation might be prospective therapeutic targets. Many nutrients must also be delivered into organelles like the apicoplast, which has numerous critical metabolic processes unique to plants or bacteria and depends on a continuous flow of raw materials from the parasite cytoplasm. However, the molecules controlling these transport mechanisms are still largely uncharacterized, and further investigation into this area may help identify a very important target for the creation of antimalarial drugs.

Furthermore, the parasite that relies on a Ca++ signaling system to carry out its essential tasks faces an intriguing challenge due to the survival of the parasite under low cytosolic Ca++ concentration in RBC. After membrane invagination during erythrocyte invasion,

Gazarini et al. propose that the Ca2+ ATPase on the RBC membrane, which pumps Ca++ across the plasma membrane toward the extracellular space, turns toward the parasite and transports Ca++ from the host cytosol across the PVM and into the PV, allowing a Ca++ gradient to form and enabling Ca++ signaling within the malaria parasite. This suggests that the PVM has to be modified in order to precisely target the RBC Ca++ ATPase as a possible site of intervention in the management of malaria.

Trafficking in Proteins

Because host cell proteins have extremely limited access to the PV, there is active unidirectional trafficking of parasite proteins across the RBC cytosol and the host cell plasma membrane in addition to their own plasma membrane's boundaries. About 5% of the parasite's genome-encoded proteins are exported into the cytoplasm of the host cell. A recent gene knockout screen revealed that about 25% of these exported proteins were implicated in crucial blood-stage survival roles, and some of these proteins on the surface of erythrocytes appeared to have virulence-associated roles, such as promoting infected cell adhesion and/or rigidity, which are the key determinants of the particular pathogenicity of this parasite. The majority of the components of a traditional vesicle-mediated secretory pathway seem to be involved in the trafficking of proteins within the parasite. However, transporting proteins outside of the parasitophorous vacuole membrane poses a significant logistical problem.

According to studies, proteins from the parasite that are meant for export reach the PPM, are released into the vacuolar lumen, or are transported across the PVM either in one step at the points where the PPM and PVM come into contact, or in two steps by being first delivered into the vacuolar lumen and then being transported across the PVM. In the latter scenario, a protein conducting channel is required to translocate the protein across the PVM, and a pentapeptide known as the Plasmodium export element or vacuolar translocation signal toward the N terminus of the protein directs traffic of proteins to locations beyond the PVM. How the PEXEL/VTS operates to export the protein to its destination is yet unknown since the PEXEL motif is cleaved inside the parasite's endoplasmic reticulum before export with processing at the conserved leucine and acetylation of the new N-terminus that starts with xE/Q/D. A translocon in the PVM known as the PTEX complex, which recognizes and translocates proteins deposited into the vacuolar space and intended for export, was recently discovered by de-Koning-Ward et al [8], [9].

However, the lack of this protein translocon machinery, even in parasites that are closely related to one another, demonstrates how difficult it is to determine its evolutionary origin and serves as an "Achilles heel" for those who produce antimalarial drugs. Numerous PEXEL-negative exported proteins, such as the membrane-associated histidine-rich protein 1, ring-exported protein, and skeleton binding protein 1, suggest that other export routes might exist. Spielmann and Gilberger contend that while mature PEXEL proteins and PNEPs lack recognized export motifs, the export route may be able to accept both kinds of proteins due to these similarities. It is now obvious that experimental data are needed to support this idea. Although, mature RBC has no endogenous trafficking machinery, some exported proteins destined for the erythrocyte cytoplasm and membrane associate with P. falciparum-induced heterogeneous structures of convoluted flotillas of flattened discs that are tethered to the RBC membrane, called the maurer's clefts, and the later play an important role in the trafficking of parasite proteins to the surface of the host cell.

The parasite exhibits astounding diversity in the kinds of proteins it exports to the MCs and in the roles those proteins perform within the MCs. Two new pathways are proposed for the export of MC resident proteins to the MCs: a PEXEL-independent pathway, in which PNEPs

may accumulate or diffuse laterally into the MCs as MCs form and "bud" out of the PVM; however, additional experimental evidence is required to confirm protein entry into the forming MCs and the needs for a "sorting signal"; and a second pathway, in which some PNEPs and PE A tubovesicular membrane network extending from the PVM is present inside the infected erythrocyte and is distinct and independent from the MCs. It is debatable whether there are connections between the TVN and MCs such that there is continuity between both compartments. Additionally, TVN features unique markers that are not present in MCs, including erythrocyte vesicle protein 1, TVN junction protein, sphingomyelin synthase, etc. Inhibiting TVN production had no impact on protein export via MCs. According to Tamez et al the parasite uses different membrane networks for macromolecule export and import. In contrast to MCs, TVN is rumored to be open to the outside of the erythrocyte surface and is thought to be engaged in macromolecular import. Since proteins generated by parasites alter the characteristics of RBCs, identifying the critical stages of variant antigen transit via the MCs is essential for the success of therapeutic treatments.

Removal from RBC

The parasite remains in the PV throughout subsequent phases like ring, trophozoite, and schizont throughout erythrocytic development. By the time the majority of the RBC hemoglobin has been depleted, the schizont generates 8–24 mature merozoites, which must breach the PV and RBC membranes in order to leave the host cell and begin a new cycle of RBC invasion. Multiple kinds of proteolytic enzymes are involved in the closely controlled process of merozoite egress from host erythrocyte. Recently, Arastu-Kapur et al identified two proteases as the main regulators of this process: the dipeptidyl peptidase 3 and the subtilisin family serine protease PfSUB1. The abundant papain-like serine repeat antigen are processed by the controlled secretion of PfSUB1 into the PV from the exoneme, a new organelle separate from the apical organelles, which then acts on membrane components of the PV and RBC to start host cell rupture . A family of cysteine proteases known as the DPAP proteases is thought to have a role in parasite egress via controlling PfSUB1 maturation. Additionally, the related DPAP1 protease plays a crucial role in the last stages of hemoglobin breakdown, and both the DPAP and PfSUB1 protease classes may be prospective targets for therapeutic intervention [8]–[10].

Malaria Infection and Severity: Host-Parasite Interactions

A high parasite burden, the ability of parasitized erythrocytes to adhere to vascular endothelial cells , uninfected erythrocytes , and platelets as well as a high parasite burden are thought to be responsible for the various clinical features of malaria, including impaired consciousness, coma, breathing difficulty, severe anemia, and multiorgan failure . The interactions between host receptors and parasite-encoded clonally variable antigens on the surface of parasitized erythrocytes have been connected to all of these events. In P. falciparum, three groups of variant genes have been identified: the repetitive interspersed family of genes, the subtelomeric variant open reading frame genes, and the var genes producing P. falciparum erythrocyte membrane protein 1. Although the rif and stevor genes seem to be closely linked, their receptors have not yet been discovered, and it has been shown that PfEMP1 is typically involved in adhesion to host receptors. The var genes are expressed during the pigmented trophozoite and schizont phases, and the resulting PfEMP1 traffic from the Maurer's clefts to the erythrocyte membrane where they are arranged into knob-like structures.

The var genes are closely controlled at the transcriptional level. A parasite has a family of 60 var genes, but only one of them is expressed at a time, producing the antigenically distinct

PfEMP1 variant. This gene also contains extracellular regions made up of duffy-binding-like, cysteine-rich interdomain regions, and C2 domains, which are tandemly arranged cysteine-rich domains. On the basis of conserved upstream regions, the PfEMP1 can be divided into three major groups with different functional and clinical significance. The binding domains for several host receptors, such as CD36, complement receptor 1, and ICAM1, have been mapped to individual DBL and CIDR domains. These host receptors include CD36, complement receptor 1, and ICAM1. The parasite may alter the antigenic and functional features of infected erythrocytes by the switching of var gene expression, which can happen at every new asexual blood stage cycle. This enables the parasite to evade immunity and change the ability to adhere. In order to circumvent the host's regular splenic clearance systems, the adhesion characteristics of parasite isolates might vary in consecutive cycles when PfEMP1 expression varies. The ability to switch between phenotypes adds another layer of complexity, and it is still unknown what variables influence which var gene is chosen for transcription in each IE.

Furthermore, it has been challenging to identify which of the three major adhesion phenotypes contributes to malaria that can be fatal, despite the fact that the cell adhesive properties of IEs cause microvascular obstruction, metabolic disturbances, and the release of harmful inflammatory mediators. Even though every P. falciparum isolate sequesters, not every infection results in a life-threatening illness, and recent data point to the possibility that geographic variation in adhesion phenotypes may result in severe malaria, which may be caused by variations in host immunity and malaria transmission levels. Additionally, the ability of P. falciparum parasites to express var2csa selectively in pregnant hosts mediates IE attachment to chondroitin sulfate A expressed on the placenta's syncytiotrophoblasts, which is linked to intrauterine growth restriction, miscarriage, and premature delivery. However, after several pregnancies, acquired immunity to CSA-binding PfEMP-1 molecules protects both the mothers and their fetuses, providing strong evidence that variant antigens contribute to the parasite's immune evasion and virulent mechanisms. Mothers in their first pregnancy are particularly susceptible to these complications. Beyond the topic of this research, IEs are also known to attach to a number of immune system cells, which has significant immunological ramifications.

Malaria and Red Blood Cell Polymorphisms

A lot of polymorphisms in the genes encoding certain erythrocyte surface proteins, hemoglobin, and other immune effectors have been naturally selected in the human genome as a consequence of the strong evolutionary pressure that malaria has placed on it. Although homozygosity for these same genetic variants can occasionally cause other diseases that might have otherwise disfavored their retention, in areas of the world where malaria is endemic, the combined effects of very high infection rates and deaths primarily caused by the disease in young children favor selection and retention of infection-resistant genetic variants. Erythrocyte genetic variations that alter invasion, replication, and/or clearance of iRBC have a significant impact on infection. Red cell glycophorins, band 3, duffy negative, CR1 variations, and ABO group are examples of naturally occurring red cell variants that are defective in certain surface proteins essential in parasite invasion and provide protection against severe malaria. The ABO Glycosyltransferase, a branching enzyme that converts the H precursor into the main blood types A, B, and O, respectively, is of special importance. It does this by adding galactose, N-acetylgalactosamine, or neither to it. Loss of ABO GlycosylT activity results in the "O" blood group, and functioning ABO enzymes have been linked to a higher risk of developing severe malarial anemia, suggesting that the O blood group provides some level of malaria protection.

CONCLUSION

Malaria has not yet been eradicated despite efforts in this direction. Because malaria-related pathology is only present in the blood stage of infection, it is crucial to comprehend host-Plasmodium interactions at this point in order to develop novel intervention techniques for treating severe malaria. Understanding these interactions may result in the development of novel therapeutic approaches based on the impairment of the import of essential nutrients from the extracellular milieu to Plasmodium that are not readily provided by the RBCs, the blocking of parasite trafficking of proteins to the host cell surface that are crucial for antigenic variations and evading host defense mechanisms, or the development of interventions to reverse the adhesion of infected RBCs to other human cells and inhibitors of proteases that are important for antigenic variations. Additionally, the erythrocytic stage of the illness will benefit greatly from further research into RBC polymorphisms and the molecular mechanisms through which these variations exert their protective effects.

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CHAPTER 10

A COMPREHENSIVE REVIEW ON PRIMATES' HOST-PARASITE EVOLUTION

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ABSTRACT:

Evolutionary processes allow organisms to adapt to their surroundings. Abiotic elements and living things both make up environments. Many times, two or more species interact over many generations and mutually adapt to one other's evolutionary changes. Such coevolutionary processes may be seen in mutualistic and antagonistic systems, including partnerships between predators and prey and hosts and parasites. We are now seeing this process with SARS-CoV-2. Coevolution often leads in an "arms race" between viruses and hosts and may dramatically impact the virulence of infections and hence the severity of infectious illnesses. Additionally, it may result in co-speciation, which would provide congruent phylogenies between, for instance, the host and the parasite. Primates, including monkeys, are not an exception. They serve as hosts for several infections that have influenced not just the immune system of primates but also numerous ecological and behavioral adaptations. Numerous primate species, including humans, are most certainly infected by these infections, which may also cause serious illnesses. The importance of cophylogenetic studies as a coevolution indicator is highlighted in this short summary of broad characteristics of the coevolutionary process in its strict definition.

KEYWORDS:

Bacteria, Infectious Disease, Phylogeny.

INTRODUCTION

Lemurs, galagos, and lorisids make up the Strepsirrhini suborder of the mammalian order of primates, whereas tarsiers, monkeys, and apes which include humans make up the Haplorhini suborder. Like any other living thing, primates serve as hosts for a variety of other species, such as gut or skin bacteria that live on or in their environments. Relationships between these organisms and the host can be categorized as mutualistic, commensalist, or parasitic, depending on the effects on the host's Darwinian or evolutionary fitness. The host's cellular activities are used by parasites to live and develop in or on it. The fitness and health of the host may be significantly harmed by viruses, bacteria, protozoa, helminths, and arthropods. A parasite may be categorized as a pathogen if these harmful effects can make the host ill. Even yet, certain parasites, such as brood parasites like the cuckoo, have a significant negative impact on the fitness of the host. In the review that follows, pathogens will take center stage, and the words parasite and pathogen will be used rather interchangeably [1], [2].

Since at least anthropoid primates share a similar physiology, pathogens are a natural component of wild primates' habitats. This means that many of these pathogens have the potential to spread across species boundaries fairly easily, including transmission between humans and nonhuman primates and vice versa. Given that there is contact, a transmission is more probable the closer the evolutionary ties among potential host species are. Even

pathogens that only infect a certain species of monkey may develop into ones that only infect humans. Our knowledge of the coevolutionary processes between hosts and parasites in primates is currently restricted, as it is in other animals. Like other creatures, primates have developed in many habitats, which provide a variety of difficulties for their survival and procreation. Because of genetic and phenotypic heterogeneity among people, some individuals have more children than others. They are more Darwinian fit and their corresponding alleles propagate across succeeding populations. As a result, these environmental factors act as selecting pressures, and the offspring of the individuals are thus considered to be more suited.

Abiotic and biotic components make up an ecosystem. Because interactions with other taxa are common and an essential component of the environment, organisms do not exist in isolation. Animals consume plants or other animal species, engage in resource competition, are endangered by predators, and are parasitized. They serve as the home for billions of microorganisms that are commensals, mutualists, or parasites that live on and within their bodies. To put it another way, organisms change as a result of their interactions with other species. These interactions are categorized in accordance with the fitness consequences they may have on the involved species. Individuals of both interacting species profit from their interaction when there is mutualism; however, when there is competition, both species suffer fitness losses; and when there is predation or parasitism, an individual of one species gains fitness at the expense of an individual of the other species [3], [4].

Commensalism happens when one species improves its fitness without having any negative effects on the other, and often, interactions between species are merely neutral and have no impact on the fitness of either species. A characteristic change in the first species or host is often the result of an evolutionary reaction to an evolutionary change in a second species, such as parasites and diseases. This indicates that selection acting on one population modifies gene frequencies in the other population, which again changes gene frequencies in the first population, and so on. Coevolution in a strict sense has been defined as such reciprocal evolutionary changes in two or more species.

DISCUSSION

In research on the impact of secondary chemical compounds in plants acting as defensive chemicals on herbivore butterflies, Ehrlich and Raven were the first to use the term "coevolution." However, Charles Darwin has previously made reference to the development of blooming plants and their pollinators, insects. He expanded on this concept and hypothesized that there must be a pollinator suited to the 30 cm-long nectar spur of the Malagasy star orchid . A pollinator was found and named forty years later as the Morgan's sphinx moth, which does indeed have a proboscis that is around 30 cm long. Figs and the fig wasps of the family Agaonidae, which serve as their pollinators, exhibit another fascinating mutualistic coevolution. There are about 750 different kinds of figs, and each one has a unique type of pollination wasp that responds to the volatile signals.

When used strictly, the term "coevolution" refers to paired interactions between species, such as when two species, A and B, exhibit reciprocal trait changes. A species an evolutionary change is followed by a species B evolutionary change, which in turn leads to a second species an evolutionary change, and so forth. However, the situation is often more complex since different species interact with one another, which may lead to diffuse coevolution, or the mutual effect of numerous interacting species. As an example, many plant species interact with various pollinator species. In contrast to other related Hymenoptera, honeybees visit and pollinate a variety of plant species. Additionally, interactions between different species may

be seen in relation to how seeds spread in plants. A large group of frugivorous animals within an ecological community have the capacity to distribute seeds. In order to co-adapt their fruit and seed traits to the whole guild or just a few specific specialist frugivores, plants. On the other hand, frugivores may co-adapt to specific fruit and seed traits. Diffuse coevolution, which may also be the norm when thinking about primates and their diseases, is the evolutionary interaction of numerous species that have impacted one another in certain reciprocal evolutionary ways.

In terms of pollination, there is some selection pressure on the plant's side to ensure that the pollinator keeps to the same plant species while transporting pollen from one bloom to the next. This is in contrast to seed dissemination. Compared to the seed distribution system, the pollen delivery system must be more exact. As a result, co-adaptations between plants and pollinators are closer than those between plants and seed dispersers. Mutualistic interactions include those between plants, pollinators, and seed dispersers as well as the capacity of folivorous primates like colobine monkeys to digest foods with a high fiber content with the assistance of their gut bacteria. The development of a predator-prey relationship, on the other hand, is an example of hostile interactions. Because a predator's prey species develops to move faster than it does, the predator is under pressure to sprint even faster. The speedier predator causes the prey species to develop a defense mechanism, and so on.

A positive feedback loop may be created by such processes of adaptation and counter-adaptation until benefits exceed costs or a physical or energy threshold is reached. Hosts and parasites may coevolve in a similar way. Such antagonistic processes may lead to unstable runaway escalations or unending "arms races," in which one side develops defenses against predation or parasitism and the other responds by developing more advanced weapons or strategies to get around those defenses and strategies. The brood parasitism of the common cuckoo is an intriguing instance. Here, it is possible to see how the cuckoo is attacked, mobbed, and killed by the host species. Common predators of cuckoo hosts include Eurasian sparrowhawks, which adult common cuckoos resemble in appearance. It is believed that cuckoos developed this mimicry to defend themselves from such assaults. The red-backed shrike, a frequent host species, provides evidence that this mimicry is no longer as successful as it once was [5]–[7].

The word "coevolution" is often used to refer to evolutionary "arms races" that take place inside a single species, or even within an individual within a single host). The coevolutionary reinforcement between brain size and social system complexity in primates and cetaceans is similar to these occurrences but does not meet the formal definition of coevolution. The reciprocal adaptation between two populations or species does not occur in the cases of sexual conflict and the evolution of brain size, and in the case of viruses, the host is more like a kind of habitat for the parasite, and its immune system acts as selection pressure on the parasite population. The individual host, on the other hand, does not and obviously cannot react by going through an evolutionary shift. The classification of viruses as obligatory intracellular parasites is important to emphasize.

Parasites and Coevolution

Coevolutionary cases involving parasites and their hosts are fascinating. However, since selection may occasionally only affect one partner, host-parasite interactions do not always meet the requirements for coevolution. Invading a tiny population of a host species, for instance, a parasite may force selection and genetic change on the host population without changing itself. Additionally, host mortality might be quite high if a novel parasite infects an unprepared host population. For instance, the unintentional introduction of a fly whose larvae

feed on the blood of bird nestlings resulted in a death rate of 55% among Galapagos finch nestlings. Similar to this, the spread of infections from humans to populations of naive great apes, such as pandemic human viruses, poses a serious threat to these endangered species and is thus of tremendous conservation concern [8], [9].

As in predator-prey systems, the threat of parasite infection acts as a powerful selective force, and evolutionary "arms races" are frequently seen as hosts develop increasingly complex physiologic, behavioral, and immune defenses while parasites attempt to overcome this resistance. Because parasites may lead to frequency-dependent selection of uncommon genotypes, antagonistic coevolution between hosts and their parasites has been hypothesized as a key driver of the genetic diversity present in populations. When a parasite infects a population of hosts, it mostly comes into contact with hosts that have the most prevalent genotypes in the population and adapts to those genotypes. They may not be able to infect hosts with other genotypes if they are evolved to one host genotype, making hosts with unusual genotypes more resistant. In the wild, parasites are thought to follow certain host genotypes. Since infections by definition diminish the fitness of the host, the previous most common genotype will become less common, while the frequency of hosts carrying the initial unusual genotype would rise and the parasite will adapt to the now-dominant genotype. It is thought that this kind of frequency-dependent selection by parasites is crucial for maintaining immune system polymorphisms and suggests the possibility of coevolution. There is some evidence, at least in humans, that early exposure to certain parasite types alters the immune system of the host in a manner that lowers the likelihood of allergies and autoimmune illnesses. This does not, however, negate the fact that infections generally have a detrimental impact.

It is well recognized that several parasites have various routes of transmission, including both sexual and non-sexual contact. Syphilis and yaws, vaginal and face herpes, pubic and head lice, and genital and cutaneous warts are examples of conditions when this is the case. It's possible that a single genotype has several channels of transmission or that the parasite population comprises many genetic strains that are each uniquely tailored for a certain method of transmission to explain the duality in their mechanisms of transmission. The complexities of the adaptive immune system, which has developed in vertebrates as a highly sophisticated and multi-layered defensive mechanism, are beyond the purview of this study. As a precaution to increase genetic diversity within host populations, which would make it harder for parasites to attack a host, it is even hypothesized that the risk of parasite infection drove local adaptation and the prevalence of sexual reproduction over the more effective asexual reproduction [10]–[12].

Additionally, the commensal or mutualistic species typically outnumber the parasites in the host's "meta-organism", contributing to parasite defense and immune system regulation. This can make coevolutionary processes rather complex because here, the large number of species involved makes coevolution more diffuse. If numerous parasite species infect a host population, similar broad coevolution may take place. The pace and type of host-parasite coevolution are influenced by exposure to various parasites, according to studies using bacteria and associated bacteriophages. if advantageous symbionts coevolve with their hosts and if and how microbial parasites may have evolved into protective symbionts are intriguing questions in this context.

Virulence

According to evolutionary theory, hosts change in ways that reduce the fitness cost of infection, while parasites change in ways that optimize fitness by using the host as an

ecological resource. The impact of parasites on the fitness of the host might vary in intensity. Some parasites, like lice, may only suck a few drops of blood from the host, but others, like the viruses that cause Ebola and yellow fever or the bacteria that cause anthrax in nonhuman primates, may result in significant death. Therefore, the virulence of parasites varies by definition. But virulence varies not just between parasite species but also between host species. Different host species may exhibit varying levels of pathogenicity from the same parasite. For instance, the Macacine alpha-herpes virus 1 in different macaques induces symptoms like those of the human herpes simplex virus. But in people, the same virus may cause a catastrophic condition of the central nervous system with a mortality rate of around 70%. Immunodeficiency viruses are some further examples. While the human immunodeficiency virus causes AIDS in humans and the simian immunodeficiency virus of chimpanzees is pathogenic in free-ranging chimpanzees, other closely related SIVs, e.g., in African green monkeys or drills, replicate efficiently in their natural hosts, without causing clinical symptoms. These findings imply that host characteristics that are species-specific, such as interspecies variations in immune response, rather than intrinsic parasite components, control the parasite's pathogenicity.

The coevolutionary interaction between the parasites' reproductive and survival tactics and the various levels of the host's defenses is most likely what leads to differential virulence. Additionally, parasites must go from one host to the next, and high virulence may balance off high transmissibility, as in the case of a sexually transmitted parasite that kills the host before the next sexual encounter can take place. Therefore, one may anticipate that sexually transmitted parasites would secretly infect their hosts by giving off little symptoms of illness. Byproducts of cryptic infections may also include a tendency toward reduced pathogenicity. Therefore, having a virus with high virulence like the Ebola virus may not be the best course of action for HIV. The course of parasite infections in people and groups is influenced by trade-offs between virulence and transmissibility. HIV provides more support for the virulence trade-off paradigm. It is most likely due to the apparent conflict between the two levels of selection pressure on the virus why some infected host persons get AIDS swiftly while others stay healthy without therapy for years. On the other hand, sexual behavior and mate choice may be impacted if a sexually transmitted pathogen causes symptoms that can be perceived by potential sexual partners, as was the case with olive baboons infected with Treponema pallidum when females avoid mating with males showing visible signs of infection [13], [14].

When a novel parasite first enters a population, its virulence may initially be high. However, when the host adapts over time due to coexistence, the parasite's virulence will eventually decrease. However, the result of the "arms race" is not necessarily thus. Some genotypes of parasites will have a selection advantage since most parasites can replicate within a host. They make it possible for certain people to outsmart the host's defenses, reproduce more successfully than others, and grow better at making use of the host's resources, which means that they will likely have a detrimental impact on the host's fitness. They will increase in virulence. SARS-CoV-2, a coronavirus that produced a pandemic, is an intriguing case where one can watch the development of a virus. Because SARS-CoV-2 transmits over the course of infection, long before severe or fatal effects arise, transmission and pathogenicity are essentially separated. As a result, it is also hard to say for sure whether future viral variations would be more or less virulent than the widely spread omicron forms that are present today. It is uncertain whether future SARS-CoV-2 variations will largely emerge from omicron variants or from phylogenetically distant lineages, becoming more virulent and/or simpler to transmit. This is because SARS-CoV-2 evolution is unpredictable [15], [16].

The relationship between the myxomatosis virus and its rabbit hosts is one well-researched example of host-parasite coevolution. The virus was particularly virulent when it was first introduced in Australia to reduce the rabbit population; it killed more than 99% of infected rabbits with a mean survival duration of less than two weeks. After some time, the rabbit population was infected by less dangerous virus strains, which quickly spread. With an average survival duration of 2.5 to 4 weeks, the death rate decreased to 75-90 percent. The virus had reached a degree of virulence that was intermediate, most likely as a result of trade-offs between virulence and transmissibility.

In order to provide enough time for transmission to new hosts, virus population densities should not be too low and host survival times shouldn't be too short. The rabbits developed more resistance as the virus became less virulent, but the resistance remained insufficient, perhaps as a result of the population's low genetic diversity. SIV in African green monkeys is one instance where the phylogenies of parasites and hosts closely match. The presence of a monophyletic clade of SIV lineages in each of the five investigated species of green monkeys points to close coevolution and codivergence between the host and the parasite.

CONCLUSION

Understanding host-parasite relationships and, by extension, infectious illnesses in primates, requires an understanding of coevolution. Comparative examinations of the phylogenies of parasites and their hosts may provide light on host switches and the epochs during which parasites and hosts interacted, even if co-adaptation processes are sometimes challenging to investigate. This may aid in developing hypotheses about how parasites move within and across populations and species as well as helping to understand variations in virulence of the same or similar parasites in various hosts. A study that compared the phylogenies of virus families with those of their eukaryotic hosts showed that although host and parasite phylogenies are typically not strictly correlated, there is frequently a tendency for congruence between the two. The research also showed that the studied viruses almost all exhibit cross-species transmission.

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CHAPTER 11

DISEASES CAUSED BY PARASITES: IMMUNOLOGY AND CELL BIOLOGY

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ABSTRACT:

The emphasis of this special issue of the Journal of Biomedicine and Biotechnology is on international studies of the immunology and cell biology of parasite illnesses. This issue has essays from world-renowned authors as well as some of the hottest subjects in parasite immunoparasitology and parasite cell biology, with a focus on parasites that cause major morbidity and death globally. Why was this special issue created to display these works, I hear you ask? There are three primary causes for this; the first is that this study area has advanced significantly over the last ten years, as shown by statistics indicating an increase in the share of global scientific publications of about 100% over the same period. Notably in the subject of parasitology, the number of Ph.D. scientists conferred globally and notably in developing nations has increased dramatically in recent years. Notably, though at varying rates, more and more scientists in both wealthy and developing nations are working in this area. In reality, during the last ten years, broad attempts have been made to advance the development of this research in undeveloped nations, mostly because parasites are in fact a serious health concern.

KEYWORDS:

Biology, Cell, Immunology, Parasites, Protozoans.

INTRODUCTION

The second reason is that various parasites, which put a quarter of the world's population at risk or expose them to them, harm their health. Ecology, environment, economics, and social status are particularly conducive to the emergence of a broad range of parasitic illnesses in undeveloped nations. Additionally, globalization and the rise in migration between wealthy and impoverished nations encourage the global spread of several parasitic illnesses. Along with the migration, it is undeniable that human-caused global climate change is causing parasitic illnesses to become endemic in formerly nonendemic regions. As a result, a large number of academic institutions and scientists worldwide are seriously interested in and active in parasitology research. The third reason is that despite significant efforts made by several institutions, we have reached the first decade of the twenty-first century without developing a reliable vaccination against any parasite illness that affects humans. This fact encourages us to do more thorough study so that we may understand our opponents better, eventually find a suitable cure for those who are afflicted with these viruses, and, of course, avoid infecting our children and future generations. None of these, however, will be achievable without research and financing to increase the depth of information that can be used to discover new treatments or vaccinations [1], [2].

Here, we've chosen a number of articles, including reviews and original research, on various important parasitic diseases that affect people all over the world. These articles cover

everything from basic biology, genetics, and pathology to vaccine development and the immune system's reaction to protozoa and helminths. In order to maintain the caliber of JBB, it is crucial to mention that around 35% of the papers submitted for this special issue were rejected; also, it is significant that more than half of the articles included in this special issue had previously been referenced at least once in 2010.

Parasites of protozoans

Studies on some of the most significant protozoan illnesses, including malaria, amebiasis, Chagas disease, African tripanosomiasis, toxoplasmosis, and leishmaniasis, may be found in this special issue. Travelers from all over the globe are still impacted by Entamoeba histolytica-caused amebiasis, which is still a health issue in Asia and Latin America; in their review, I. Wong-Baeza et al. "The role of lipopeptidophosphoglycan in the immune response to Entamoeba histolytica" describe the importance of lipopeptidophosphoglycan, which is recognized through TLR2 and TLR4 and leads to the release of cytokines from human monocytes, in the immune control of this infection that mainly cause diarrhea and hepatic abscess; thus lipopeptidophosphoglycan is a possible candidate molecule to develop a vaccine against amebiasis. Knowing this parasite's genes and how they work is essential to understanding it. In their first article, I. According to López-Reyes et al.'s study, "Detection of the endosomal sorting complex required for transport in Entamoeba histolytica and characterization of the EhVps4 protein," endosomal sorting complexes necessary for intracellular transport, known as EhVps4, are present and expressed in E. coli and are associated with increased phagocytosis and virulence [3], [4].

DISCUSSION

Toxoplasmosis, which is brought on by the protozoan Toxoplasma gondii, is another parasitic illness that is quite common. These parasitic infections are prevalent and deadly in immunocompromised people, such as HIV-positive individuals. With a focus on the toll-like receptors (TLRs) that can be activated by the parasite itself and emphasizes the complex interactions with the intestinal microbiota that worsen the disease during enteric infection, E. Y. Denkers' "Toll-like receptor initiated host defense against Toxoplasma gondii" reviews recent developments in innate immunity to this parasite. Additionally, a significant element in toxoplasmosis is immune control, particularly E. The study "Reduction of Foxp3+ cells by depletion with the PC61 mAb induces mortality in resistant BALB/c mice infected with Toxoplasma gondii" by P. Tenorio et al. demonstrates that T regs ablation causes significant mortality in otherwise resistant animals. But Y, on the other hand. Sanchez et al.'s study "The unexpected role for the aryl hydrocarbon receptor on susceptibility to experimental toxoplasmosis" revealed the AHR's first-ever involvement in a parasitic illness. They claim that mice with genetic deficiencies in AHR died from T more quickly. AHR has a part in toxoplasmosis immune modulation because of the enhanced inflammatory cytokine response that was linked to the gondii infection.

Another parasitic ailment that is prevalent across the globe is leishmaniasis. These parasites need a vector, hence the sand fly bite serves as their means of transmission. Its distribution over all continents is a result of several species. Here, we've chosen a collection of publications that demonstrates the starkly diverse sides that these infections take on. The initial stage of the infection is crucial, and neutrophils seem to have a significant role in influencing the kind and size of the L. "Charmoy et al.: The prominent role of neutrophils during the initial phase of infection by Leishmania parasites." In accordance with this, M. Cummings et al. "Cytokines and their STATs in cutaneous and visceral leishmaniasis" discuss the critical role of signal transducer and activator of transcription (STATs) during

cutaneous and visceral leishmaniasis. Intriguingly, the first discovery by S. revealed that Leishmania infection may modify host cell signaling in order to survive. Nevertheless, more efforts are being made to develop fresh methods to obtain useful vaccines (Ramrez et al.: BALB/c mice vaccinated with Leishmania major ribosomal proteins extracts combined with CpG oligodeoxynucleotides become resistant to disease caused by a secondary parasite challenge), as well as development of new drugs after detecting high resistance to the old drugs.

Tropical illness malaria, which kills over a million people a year, is brought on by protozoan parasites of the genus Plasmodium and spread by Anopheline mosquitoes that feed on blood. There is currently no effective vaccine for malaria. The output of J. A multifactorial mechanism in the higher antimalarial activity of -C-GalCer is shown by Schmieg et al., proving that -C-GalCer possesses an antimalarial activity. When administered in vivo, -C-GalCer causes dendritic cells (DCs) to mature more slowly and mouse invariant V14 (V14i) NKT cells to respond more proliferatively, both of which may in part explain -C-GalCer's greater in vivo activity. In a similar vein, A. G. and Kuehn. In his overview of current research on gametocytes' roles in mosquito transmission, Pradel's "The Coming-Out of Malaria Gametocytes" focused particularly on the molecular processes underpinning gametocyte activation and emergence from the host erythrocyte during gametogenesis. The telomeres and subtelomeric portions of Plasmodium falciparum's chromatin were poorly understood until relatively recently, which is why R. The article "Telomeric heterochromatin in Plasmodium falciparum" by Hernandez-Rivas and colleagues discusses the telomeric heterochromatin in P. falciparum and makes the case that this structure may be crucial for telomere functions like the silencing of the var gene family, which is linked to the parasites' cytoadherence and antigenic variation. N. The study "The lipid moiety of haemozoin (Malaria Pigment) and P. falciparum parasitised red blood cells bind synthetic and native endothelin-1" by Basilico et al. focused on the interaction between a malaria pigment of P. falciparum parasitised red blood cells and a synthetic and native endothelin-1 [5]–[7].

Their research might be used to better understand the effects of parasite sequestration in severe malaria. According to K, the protective immunity induced by tomatine adjuvantation to a key pre-erythrocytic malaria vaccine candidate is mediated via the production of IFN- by CD8+ T cells. In their study, "Tomatine adjuvantation of protective immunity to a major pre-erythrocytic vaccine candidate of malaria is mediated via CD8+ T Cell release of IFN-," G. Heal and Taylor-Robinson come to the conclusion that further research into the role of tomatine as an adjuvant in the development of malaria vaccines is necessary. Finally, M. engaged in similar adjuvant action. Legorreta-Herrera et al.'s "Pretreatment with Cry1Ac protoxin modulates the immune response, and increases the survival of plasmodium-infected CBA/Ca mice" demonstrated that the pretreatment with Cry1Ac protoxin modulates the immune response of the host and increases the survival of Plasmodium-infected mice, and pointed out that by understanding how to boost innate immunity to Plasmodium infection should lead to immunological based intervention strategies.

The protozoan Trypanosoma cruzi and T are responsible for trypanosomiasis. brucei, which are spread by insects called vectors. Here, we include a number of original publications and reviews that address both American and African trypanosomiasis, generally known as Chagas disease and sleeping sickness, respectively. Global warming and migration both increase the risk of contracting these illnesses in humans and domestic and wild animals. C. Utilizing mass spectrometry, M. Atyame Nten et al. "Excreted/Secreted proteins from trypanosome procyclic strains" described over 500 proteins secreted by T. brucei and hypothesized a crucial part for such a secretome in the parasite's pathogenicity. Similar to this, R. A method

for experimentally identifying conserved secreted proteins in the same parasite was devised by M. Corrales et al. in their paper, "An experimental approach for the identification of conserved secreted proteins in Trypanosomatids." Six key surface proteases that are encoded within the partly sequenced T have been described by V. Marcoux et al. in their study "Characterization of Major Surface Protease Homologues of Trypanosoma congolense" in the case of African trypanosomiasis. Genome of Congolense.

We have two fascinating studies that deal with the expression and regulation of genes in these protozoans since it seems to be a significant area for the virulence of these parasites: "S. Gene expression in trypanosomatid parasites by Martnez-Calvillo et al. and "C. In line with this, Hernández-Osorio et al.'s "Improved method for in vitro secondary amastigogenesis of Trypanosoma cruzi: morphometrical and molecular analysis of intermediate developmental forms" created a method to analyze the intermediate forms of T morphometrically and molecularly. cruzi in a dish. Finally, we have one original paper and one review discussing immunological regulation during trypanosomiasis. T. N. Baral provided a very thorough analysis of the immunobiology of African trypanosomes in "Immunobiology of African Trypanosomes: Need of Alternative Interventions" and suggested a different direction for research to advance methods for treating and preventing trypanosomiasis. B. However, Espinoza et al.'s study, "Mexican Trypanosoma cruzi I strains with different degrees of virulence induce diverse humoral and cellular immune responses in a murine experimental infection model," demonstrates how various T. Cruzi exhibit varied levels of virulence and cause extremely variable cellular and humoral immune responses, which explains some of the difficulty in developing a viable therapy or vaccine for this parasitic illness [8], [9].

Hematophagous vectors, which are significant biological agents, are the primary means by which many protozoan diseases are spread. S. provides information on the function of cysteine-free proteins in the immunobiology of illnesses carried by arthropods. Mejia et al. describe their results in the context of protein structure and function, antigenicity and immunogenicity, and host-parasite connections in their article "Cysteine-Free proteins in the immunobiology of arthropod-borne diseases." Another piece by R. "Differential midgut attachment of Leishmania (Viannia) braziliensis in the sand flies Lutzomyia (Nyssomyia) whitmani and Lutzomyia (Nyssomyia) intermedia" by P. Soares et al. demonstrates how L. braziliensis have a variably variable adhesion to the midgut of several species of its sand fly vector, Lutzomyia. In a letter written especially for this report and in the same context, C. According to R. Alves et al., "The vectorial potential of lutzomyia (Nyssomyia) intermedia and lutzomyia (N.) whitmani in the transmission of leishmania (V.) braziliensis can also be related to proteins attaching" may also be important for this infection.

Infections with helminths

Multicellular organisms known as helminths have the ability to invade practically any tissue in their hosts. Nematodes (roundworms), trematodes, and cestodes (flatworms) are the three categories into which they are divided based on their mature forms. The immune response to these diseases is particularly difficult because of how large they might grow. Millions of individuals throughout the globe are infected with worms, which range in virulence from hardly offensive to very harmful and, in some instances, life-threatening for humans. We have chosen research pieces and cutting-edge reviews for this special issue that span all three major types of helminths. Alveolar echinococcosis is caused by the bacterium Echinococcus multilocularis. The review by D. B. Burberry and A. Gottstein discusses the immuno-modulatory mechanisms involved in the pathology and defense against the larval stage (metacestodes) of this parasite in "Echinococcus multilocularis and its intermediate host: a model of parasite-host interplay" and suggests the use of cytokines like interferon and

specific antigens to treat patients in the future in order to reduce the immunopathology associated with alveolar echinococcosis and/or to prevent.Taenia solium received a gift from A. The study by Landa et al., "Release of glycoprotein (GP1) from the tegumental surface of Taenia solium by phospholipase C from Clostridium perfringens suggests a novel protein-anchor to membranes," demonstrates that GP1 is a novel protein-anchor to membranes when it is released from the tegumental surface of its metacestode. There was further investigation into T. The part that sex steroids play in the parasite's metacestode stage growth is called the solium. Progesterone therapy of the parasite in vitro, in particular, causes scolex evagination and development of the same. What seems to be a progesterone receptor on the parasite mediates these actions. In their paper "Escobeda et al.: Progesterone induces scolex evagination of the human parasite Taenia solium: evolutionary implications to the host-parasite relationship," the authors note that Taenia crassiceps, a rodent parasite, uses the hormone, and that this use by the parasite may have significant evolutionary implications for the host-parasite relationship.

However, for a long time, it has been acknowledged that this parasite is a viable choice for doing research to provide more insights into the illness caused by T. solium. Readers can discover multiple articles addressing various facets of the infection by T in this issue. crassiceps. First, P. Ostoa-Saloma et al.'s research, "Budding of Taenia crassiceps cysticerci in vitro is promoted by crowding in addition to hormonal, stress, and energy-related signals," shows that T. Crowding, in addition to hormonal, stress, and energy-related cues, promotes crassiceps cysticerci in vitro. G, however, is the opposite. Escobedo et al. "A new MAP kinase protein involved in estradiol-stimulated reproduction of the helminth parasite Taenia crassiceps" demonstrate the process of discovering a new protein from a parasite's origin, a parasite MAP kinase, which may be involved in the estradiol-stimulated reproduction of this helminth parasite. The authors of the study "Substance P signaling contributes to granuloma formation in Taenia crassiceps infection, a murine model of cysticercosis" conclude by suggesting that substance P signaling may be involved in the development of granulomas and the release of proinflammatory cytokines in this infection [10]–[12].

With regards to Schistosoma japonicum. In their study, "Activation-induced T helper cell death contributes to Th1/Th2 polarization following murine Schistosoma japonicum infection," Xu et al. show how this infection causes activation-induced T helper cell death and hypothesize that S. The Th1/Th2 shift is involved in japonicum antigen-induced Th1 and Th2 cell death and favors both hosts and parasites. S. Hu et al.'s work "Anti-inflammatory protein of Schistosoma japonicum directs the differentiation of the WEHI-3B JCS cells and mouse bone marrow cells to macrophages" was the last one to create one that discovered an anti-inflammatory protein of S. japonicum (rSj16), which instructs the WEHI-3B JCS cells and mice bone marrow cells to differentiate into macrophages. Their findings showed that rSj16 influenced mouse bone marrow cells to form colonies that were more likely to descend from macrophages.

E. presents immunity against helminths, their interactions with the host, and the concomitant illnesses they produce. Greater and A. They claim that research on the immune response against helminths is very important for understanding how the host immune system and parasites interact. Chauvin, "Immunity against helminths: interactions with the host and the intercurrent infections." Finally, K represents in the work still another form of parasites and hosts that are described here. Both Rohlenová and A. "Are the reproductive efforts of cyprinid fish affecting the immunocompetence and presence of metazoan parasites in cyprinid fish?" asked imková", on the potential effects of their reproductive activities on the immunocompetence and the prevalence of metazoan parasites in cyprinid fish. On the same

subject of parasites and fish, P. The topic of T. K. Woo's book "Immunological and Therapeutic Strategies against Salmonid Cryptobiosis" is immunological and therapeutic approaches against Salmonid cryptobiosis, which is brought on by the haemoflagellate Cryptobia salmositica. A monoclonal antibody to a metalloprotease's treatment is the disease-causing agent. Even though a vaccine has been created, the only treatment that works against the infection is isometamidium chloride, and its potency is improved when it is coupled with antibodies.

In the subject of immunoparasitology, the early interactions between the immune system and either helminth and protozoan parasites or their derivatives have become a hot issue. In this issue, we provide four reviews that demonstrate the importance of innate cells such dendritic cells Similarity and variety in the way nematodes, trematodes, and cestodes activate macrophages, as well as the effectiveness of nitric oxide against various helminth infections They describe how DCs did not mature after exposure to helminth antigens, as well as how macrophages are shifted towards an alternate state of activation, and pointed out how crucial the induction of nitric oxide production is during various helminth infections. Nitric oxide and respiratory helminthic diseases.

Two further papers specifically address the function of these cells in helminth and protozoan infections; for instance, M. analyzes in depth how Peroxisome Proliferator-Activated Receptors (PPARs) mediate the inhibition of the parasiticidal response. Peroxisome proliferator-activated receptor (PPAR): balance for survival in parasite infections, in parasitic infectionsmacrophage activation, immunosuppression, and intracellular signals," by report the function of arginase in promoting susceptibility to protozoan and helminth infections is recapitulated [13], [14].

Combating Inflammation and Autoimmunity with Helminths

Accordingly, most of the helminth infections studied in more detail have shown a general mechanism that includes inhibition of the immune response, low proliferative range of T cells, induction of regulatory cells like Tregs, altered immune responses, and low proliferative range of T cells. These findings have led to a new positive point of view regarding immune modulation by helminthes. We provide a good review from Y right here. As well as T. The phrase "Parasitic helminths: new weapons against immunological disorders" by Kanazawa suggests that helminths may soon be utilized as weapons against autoimmune illnesses. Three original publications employing various helminth infections as regulators for three various diseases provide reinforcement or support for this point of view, thus A. A concurrent infection with Hymenolepis diminuta is much better at reducing the pathology associated with chemically-induced colitis than traditional treatment using corticosteroids, according to Melon's group in their study "Infection with Hymenolepis diminuta is more effective than daily corticosteroids in blocking chemically induced colitis in mice." Taenia crassiceps, a different cestode, was used in A. For the first time, it was shown by Espinoza-Jiménez et al. in their study "Taenia crassiceps infection attenuates multiple low-dose streptozotocin-induced diabetes" how this parasite, which is only found in the peritoneal cavity, was able to prevent the onset of Type 1 diabetes (T1D), which is brought on by multiple low-doses of streptozotocin. Finally, following the same progression of thoughts, P. According to Zaccone et al., "Immune modulation by Schistosoma mansoni antigens in NOD mice: effects on both innate and adaptive immune systems" demonstrates how administering soluble S. Mansoni was able to cause T cells to produce TGF-, which is crucial for both Treg expansion and the effective Th2 response required to lower T1D in nonobese diabetic mice.

CONCLUSION

With millions of victims in areas with little resources, parasitic illnesses pose a serious threat to world health. To create effective preventative and treatment plans, it is crucial to comprehend the immunology and cell biology of these disorders. With an emphasis on both protozoan and helminthic parasites, this study offers a succinct summary of the major immunological and cellular processes involved in host-parasite interactions. Innate and adaptive immune responses to parasite infections have many different facets. Neutrophils, dendritic cells, and other innate immune cells like macrophages play critical roles in the early detection and containment of parasites. For long-term immunity and parasite clearance, adaptive immunological responses which are predominantly mediated by T and B lymphocytes are crucial. However, several parasites have developed sophisticated defenses against host immunity, such as antigenic variation and host immune pathway regulation.

Both parasite and host cell cellular interactions are complex. Plasmodium, Trypanosoma, and Leishmania are protozoan parasites that alter host cell signaling pathways to create intracellular habitats and evade immune detection. Helminths like Schistosoma and filarial worms, in contrast, influence host tissue reactions to produce an environment that is advantageous for their survival. For the creation of cutting-edge treatments, vaccines, and diagnostics to fight parasite illnesses, a thorough knowledge of these immunological and cellular mechanisms is essential. This review emphasizes the necessity for multidisciplinary research to address these complex and deadly illnesses by highlighting current advancements and difficulties in the area.

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CHAPTER 12

THE MOLECULAR IDENTIFICATION OF INTESTINAL PROTOZOANS IN AN URBAN POPULATION: ONE HEALTH APPROACH TO ZOONOTIC PARASITES

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ABSTRACT:

The brown or Norway rat, Rattus norvegicus, is the most prevalent animal in metropolitan environments, where it coexists closely with people. The importance of Norway rats as a zoonotic parasite reservoir in urban areas has essentially gone unnoticed among illnesses transmitted by rodents. We aimed to detect and quantify the zoonotic intestinal protozoans in an urban population of R while taking the parasitic disorders in the One Health approach into consideration. In the Spanish city of Barcelona, norvegicus. In the winter of 2016–17, we examined the presence of ZIP in 100 rats caught in parks and the city's sewage system. Through the use of a multiplex PCR, the protozoans were molecularly identified. We also looked at whether any of the discovered species were co-infected. Significant sewage prevalencies of Blastocystis, Giardia duodenalis, and Cryptosporidium spp. were found in four ZIP codes. There were also some co-infections among the identified ZIP. Our results provide significant support for the reservoir function of ZIP that Norway rats play in cities as well as the potential role of rats as sentinels of zoonotic parasites impacting people in urban environments.

KEYWORDS:

Health, Protozoans, Parasites, Urban Environments.

INTRODUCTION

Numerous zoonotic pathogens are hosts of rodents. Humans can contract zoonotic diseases either directly that is, when the infectious agent contaminates the environment and human contact with contaminated hands, food, or water or indirectly by way of an arthropod vector that has been infected by the rodent itself. In rural or remote settings, rats play a significant role as possible zoonotic parasite reservoirs. These can be either protozoa, helminths or potential co-infections among them in an urban population of R in accordance with the One Health idea. Norvegicus, to which people are exposed, in the city of Barcelona, Spain. Only taking into account the sewage system, it has been estimated that there are 0.16 rats per person in Barcelona. 1,636,762 people were reportedly living in Barcelona as of 2018. Consequently, the sewage system of Barcelona is home to roughly 262,000 rats. Calculating the estimated number of infected rats for each ZIP will be made possible by knowing the approximate number of rats in sewers [1], [2].

Hundred R. norvegicus were analyzed to determine the intestinal protozoan parasites present. Eighty-five rats were captured in the sewage system at 19 different places, and 15 rats were captured in 7 different parks, spanning 8 of the city of Barcelona's 10 distinct districts. Snap traps were utilized in the sewage system during the winter of 2016–17, and wire rat cages

were placed in parks. Live animals were put to death by being exposed to a CO2-rich environment. The caught rats' viscera were kept in 85% ethanol until they were later studied.

Molecular Procedures

Rat intestinal contents were collected, stored in 70% ethanol, and then studied. By centrifuging the samples for 5 minutes at 2500 rpm in Midi, the samples were filtered and concentrated. This method is a clean and effective way to concentrate helminth eggs, larvae, protozoan cysts, and oocysts in feces without the use of ether or ethyl acetate. In order to extract stool DNA for PCR testing, a portion of the concentrated sample was maintained at 80 °C. The QIAamp DNA Stool Mini Kit was used to extract DNA from 200 mL of the fecal concentration in accordance with the manufacturer's instructions. The AllplexTM Gastrointestinal Panel-Parasite Assay was used in multiplex PCR to detect and identify intestinal parasites. In this panel, small subunit ribosomal RNA genes are amplified in order to identify human protist parasites such Giardia duodenalis, Entamoeba histolytica, Cryptosporidium spp., Blastocystis hominis, Dientamoeba fragilis, and Cyclospora cayetanensis. CFX Manager IVD 1.6 software was used to manage the amplification on the CFX96TM Real Time PCR System in a 25 L reaction volume containing 20 L reaction mix, 8 L water without RNA, 2 L of internal control, and 5 L of DNA. Each test comprised both negative and positive controls. Positive and negative controls were included in each PCR cycle. The Seegene Viewer V2.1 software, which is designed for multiplex assays, was used to evaluate the results. According to the manufacturer's instructions, samples were deemed positive for certain parasites if the cycle threshold was 43 cycles [3], [4].

DISCUSSION

G. Blastocystis. spp., and Cryptosporidium duodenalis. Isolates that performed well on the qPCR test were next subjected to sequencing and genotyping/sub-genotyping examinations. A direct PCR test was utilized to target the SSU rRNA in the instance of Blastocystis, and the multilocus genotyping method was used to G based on sequencing information produced by PCR of the glutamate dehydrogenase and ß-giardin genes. Isolates that tested positive for Cryptosporidium spp. were examined at the GP60 locus and the SSU marker. The ability to get typeable sequences of any parasite was never conceivable. The minimal concentration of parasite DNA available as template in each of their separate amplification processes directly contributed to the restricted sensitivity of traditional PCR techniques. This issue was likely made worse by the rats' DNA degradation as a result of being kept in ethanol storage for many months between the time of capture and the molecular examination of their intestinal contents [5], [6].

Statistical Procedures

Analysis was done on the number of infected rats and the frequency of each ZIP species. Binary Logistic Regression was used to investigate the effect of rat sex and age on the ZIP prevalence using common non-parametric tests. Chi-squared test was used to determine positive ZIP connections. The threshold for statistical significance was set at p 0.05. For statistical analysis, the IBM SPSS Statistics 26 software package for Windows was utilized. Pertaining to ZIP data for R's urban population. As far as we are aware, the only norvegicus species found worldwide is Cryptosporidium spp. has been molecularly detected in New York City, Tehran, and Nishinomiya City urban regions and Giardia spp. Iran as well. In the city of Buenos Aires, Giardia cysts and Cryptosporidium oocysts have also been identified morphologically. Brown rats in Kuala Lumpur, Malaysia, have morphologically identifiable blastocystis cysts. The microsporidian species Encephalitozoon cuniculi, which was discovered in Zurich, seems to be the only ZIP in Europe that has been detected in Norway

rat cities. In the rats that were caught in the sewer system, all ZIP were discovered. On the other hand, Cryptosporidium spp. was not discovered in the rats caught in parks, despite the fact that the number of rats investigated in parks should be considered to be smaller. Regarding ZIP's general prevalence, 82 of the 100 rats examined had at least one ZIP species, showing that the Barcelona rat population as a whole does not discriminate on the basis of sex or age. The same fecal-oral transmission pathway is used by all of the discovered ZIPs, whether they have direct or monoxenous life cycles.

As a result, human Blastocystis infective parasite develops, G. together with D. Cysts of Cryptosporidium spp. and P. fragilis. Large populations of R. are present in the city's sewage system, which is contaminated by the shed in feces. Co-infections have been discovered in the research rats, and one reason co-infections may be the case is because of the shared ZIP life cycles [7]–[9].

Blastocystis

Most Blastocystis parasites are generalists and do not have a particular host. Blastocystis is estimated to infect one billion individuals, although its relevance for public health is yet unclear. There haven't been many research looking at Blastocystis infection rates in Spain. However, a retrospective observational research was conducted between 2009 and 2014 at the Vall d'Hebron University Hospital, specifically in Barcelona. A diagnosis of 418 instances with 22% of them exhibiting symptoms suggests that Blastocystis is harmful, at least in some situations. As previously mentioned, Kuala Lumpur has the sole known data on Blastocystis infection in urban Norway rats, with a prevalence of 51%. Our research shows that rats from sewers had the greatest incidence of any ZIP, at 83.5%. That indicates that there are more than 218,000 rats infected with Blastocystis exclusively in the sewage system, based on the estimated 262,000 Norway rats that are present in sewers. Although it has been said to be little, the zoonotic contribution to human Blastocystis colonization is uncertain. The data, however, substantially support R's involvement. Norvegicus as a source of infection in human blastocystis.

Duodenal giardiasis

A G. Sewers showed a prevalence of duodenalis of 37.7%, indicating that there are likely 99,000 infected rats of this species circulating in Barcelona's sewage system. The only further molecular analysis of Giardia spp. Recent research in Teheran, Iran, on urban brown rats found a higher incidence of 76% of Giardia spp. despite the fact that it was a generic identification. Rodents may have also been a factor. As indicated earlier, identifying Giardia genetic assemblages in this investigation was not achievable. Only A and B of the eight assemblages that are officially acknowledged belong to humans. Only the rodent-specific non-human assemblage G has ever been discovered in rural Norway rats in China. However, the black rat R had the human assemblage B present. Rattus, on La Palma Island in the Canary Islands.

The fact that rodents have assemblages other than the rodent-specific assemblage G suggests that domestic animals and people share a habitat with wildlife. Norway rats are always in close proximity to human waste in cities, especially those living in the sewage system. As a result, rats are probably susceptible to the same assemblages that infect people. Both assemblages A and B have been found in individuals in Barcelona. The One Health approach in giardiasis should thus take into account not just the probable reservoir function of companion animals, but also that of brown rats in urban areas, given the significant number of Giardia-infected rats identified [10], [11].

A variety of Cryptosporidium species

One of the most common causes of diarrhea in both people and animals globally is cryptosporidiasis. According to www.isciii.es, the number of cases of cryptosporidiasis in Spain specifically rose by 175% in 2018 compared to 2017. A deeper understanding of zoonotic transmission is necessary to combat the illness using a One Health strategy. Despite being two different species, C. hominis and C. Ninety percent of human infections are caused by parvum, while 19 other species have also been found to infect people. Numerous rodent species, bovids, camelids, equids, canids, felids, rabbits, etc. all serve as reservoirs. However, it is impossible to identify the species/genotypes involved without the use of cutting-edge molecular techniques. Because of this, the original identification of Cryptosporidium oocysts in animals other than humans was made on this assumption.

Remote R. Norvegicus was thought to be a C reservoir. The investigation, however, was solely based on the shape of the shed oocysts. C was found in wild rats in Europe, according to genetic investigations, among other things. People and C. parvum. Despite the fact that just four individuals were tested, Norway rats did not respond to the survey. Urban R. molecular investigations were conducted. Cryptosporidium prevalences of 38%, 1%, and 1.5%, respectively, were found in norvegicus in Nishinomiya, Tehran, and New York.

Currently, only the zoonotic species C. Moshiri and C. Wild brown rats in China and the latter also in the Czech Republic have had occultus genetically detected. In our research, we used Cryptosporidium spp. was only discovered in rats from sewers, and its incidence was almost identical to that in Japan. In Barcelona's sewers, there are more than 89,000 sick rats, which is a significant number. Similar to giardiasis, while further genetic research is necessary, R. In urban areas, norvegicus is a definite potential reservoir for human cryptosporidiosis [12], [13].

Dentenemoeba fragilis

The intestinal protozoan Dientamoeba fragilis is regarded as being ignored. It is thought to be the second most prevalent intestinal protozoan after Blastocystis, with an incidence even greater than Giardia duodenalis, and it has been recorded globally, producing human gastrointestinal symptoms. Cases involving immigrants, native-born citizens, and vacationers have all been reported in Barcelona. Although fragilis genotypes have been reported, it is unknown how they affect pathogenicity in general. There are few animal hosts of D. except humans. Gorillas, macaques, baboons, and pigs have all been recorded to have fragilis.

However, because of the large pig population globally and the increased interaction with people, only pigs are recognized as D reservoirs. Rodents have only ever served as test subjects up to now. We located D. fragilis-infected Norway rats in Barcelona's parks and sewage system, respectively. This equates to around 37,000 infected rats in Barcelona's sewers. This is the first time that R has been naturally ill. There have been reports of fragilis worldwide. Therefore, for the same reasons as pigs are, i.e. because of their enormous numbers and close closeness to people, the Norway rat should be regarded an obvious potential dientamoebiasis reservoir [14], [15].

Additional Zoonotic Parasites

The 100 investigated rats were discovered to have ZIP, but earlier research also showed that these same rats had six other zoonotic parasites, including the protozoan Leishmania infantum, the intestinal tapeworms Hymenolepis diminuta and H. nana , the oesophageal nematode Gongylonema neoplasticum , the intestinal acanthocephalan Moniliformis

moniliformis and the hepatic nematode Capillaria hepatica . Almost 249,000 Norway rats carried some kind of zoonotic parasite in the sewers of the investigated city, which means that 95% of the rats were infected with at least one zoonotic species when all zoonotic parasites, protozoans, and helminths were taken into account.

CONCLUSION

For the first time, this work uses molecular techniques to identify substantial Blastocystis, Giardia duodenalis, Cryptosporidium spp., and D. prevalences of S. fragilis as well as the presence of several ZIP co-infections in a group of R. norvegicus in a city setting. The Norway rat was also discovered to be a novel host for D. fragilis and a fresh potential reservoir as a result. R. Because of the following factors, norvegicus should be viewed as a effective reservoir of ZIP in urban areas: its population in cities is the second-largest after that of humans; in the absence of competitors or predators, rats live longer in cities than in the wild the high ZIP prevalences found, particularly in rats from sewers; Norway rats in cities live close to Brown rats have been discovered to be sensitive indicators of parasite environmental dangers to people, according to the data collected. As a result, Norway rats in urban areas might act as sentinel animals to find zoonotic parasites that impact people. We are sure that this research, along with the two before it on R. norvegicus in Barcelona will be crucial in taking into account the contribution of Norway rats to the spread of zoonotic parasites within the framework of the One Health concept. These findings need to serve as a warning to the government to step up rat monitoring and control efforts in public health initiatives.

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