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Graduation project

Assessment of Inflammatory and regulatory cytokines responses against malariae

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بسم الله الرحمن الرحيم

"أَنْزَلَ اللَّهُ عَلَيْكَ الْكِتَابَ وَالْحِكْمَةَ وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ ۚ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا"

صدق الله العظيم

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List of abbreviation

Abbreviation	Key
p.falciparum	**Plasmodium falciparum**
CM	**Cerebral malaria**
SA	**Severe malaria**
Th1	**T helper cell 1**
Th2	**T helper cell 2**
IL	**Interleukin**
IL1-ra	**Interleukin 1 receptor antagonist**
TNF	**Tumor necrosis factor**
CD8	**Cluster of differentiation 8**
NK	**Natural killer**
GPIS	**Glycosyphosphatidylinositols**
CD36	**Cluster of differentiation 36**
TLR	**Toll like receptors**
LgG	**Immunoglobulin G**
MM	**Mild malaria**
HbAS	**Sickle cell trait**
HbAA	**Normal hemoglobin genotype**
RESA	**Erythrocyte surface antigen**
MSP	**Merozoite surface protein**
EBA	**Erythrocyte binding antigen**
GLURP	**Glutamate rich protein**
AMA	**Antigens apical membrain antigen**
CSP	**Circumsporozoite protein**
PSE	**Parasite schizont extract**

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Abstract :

Malaria is caused by the protozoan Plasmodium, which is passed on to humans by Anopheles mosquitoes. Plasmodium falciparum is the most harmful of the plasmodia that infect humans. The host genetic, immune, social, and geographic influences are likely to play a part . Malaria is transmitted to humans, by the bite of female Anopheles mosquitoes. The inoculated sporozoite stage is temporary and has no pathological consequences . Cytokines are small, non-structural proteins with low molecular weights that play a complex role in regulating inflammation and immunity . innate immunity is believed to play an important role in the removal of Plasmodium from parasitized hosts. In natural conditions, the spleen eliminates the majority of the waste, although the liver has been found to serve as an additional clearance site . The presence of the parasite can be detected by dendritic cells, macrophages, gamma delta T cells, and NKT cells. Along these lines, the presence of IL-12 is required for NK cell IFN-gamma formation, and functional cooperation occurs between macrophages and NK cells during this parasitic infection . Cell-mediated responses to P. falciparum tend to be higher in HbAS individuals than in HbAA individuals . Cytokines and chemokines are elevated in peripheral blood during an acute malaria outbreak and contribute to parasite elimination . Malaria pathogenesis is complicated, as it is most certainly a combination of immunologic and nonimmunologic pathways. It is now well recognized that extreme malaria is caused by variations in a variety of tissues and organs . An significant question is whether data on human CM are consistent with the picture emerging from mouse studies. According to some studies, histopathological features observed in human CM are correlated with local development of TNF-, IFN-, and IL-1, as well as IL-10.

Introduction:-

Despite a decade of concerted interventions that have significantly decreased malaria mortality and morbidity, the disease remains a major health problem in tropical countries .(1)

Malaria is caused by the protozoan Plasmodium, which is passed on to humans by Anopheles mosquitoes. Plasmodium falciparum is the most harmful of the plasmodia that infect humans. The parasite causes the majority of the clinical symptoms of this condition at the periods where it multiplies asexually in red blood cells. (2)

Infection with Plasmodium falciparum is most serious in infants. However, only a small percentage of affected infants experience serious symptoms, which may cause severe and life-threatening illness in nonimmune persons.(3)

The host genetic, immune, social, and geographic influences are likely to play a part. Malaria is the world's most common parasitic disease, and successful prevention methods are desperately needed.(4)

One of the challenges in developing an effective Plasmodium vaccine is our current lack of understanding of defensive immunity and how it can be caused. Furthermore, the pathogenesis of two of the most serious complications of P. falciparum malaria, cerebral malaria (CM) and severe malarial anemia (SA), tend to require immune system dysregulation (5)

As a result, a deeper understanding of the processes of defensive immunity on the one hand and immunopathology on the other will offer vital hints as to how to manipulate the immune system in order to accomplish the target of better vaccinations.(6)

Malaria is transmitted to humans, by the bite of female Anopheles mosquitoes. The inoculated sporozoite stage is temporary and has no pathological consequences. Within a few minutes, it infects liver cells and enters a clinically

quiet cycle of intracellular replication. Since completing liver-stage replication, the parasite initiates blood-stage inflammation, which is the primary cause of disease.(3)

Cytokines are small, non-structural proteins with low molecular weights that play a complex role in regulating inflammation and immunity.(7) Cytokines are involved in almost all inflammatory responses in the body and play an important role in organizing immune responses between cells by binding to a number of receptors and inducing cell-specific immune responses.(8) cytokines are released by a variety of cell types, including leukocytes, which control immunity, inflammation, and hematopoiesis. About 200 cytokines have been described to date.(8)

Under pathologic conditions, these anti-inflammatory mediators may either

(1) in immune-mediated disorders, have inadequate control over proinflammatory behaviors

(2) overcompensate and suppress the immune response, exposing the host to systemic infection.(9)

Cytokines include interferons, interleukins, the chemokine family, mesenchymal growth factors, the tumor necrosis factor family, and adipokines are examples of cytokines. Cytokines are formed by all cells and evoke a response from them.(9) The net effect of each cytokine is determined by the timing of its release, the local milieu in which it functions, the presence of opposing or synergistic elements, the density of cytokine receptors, and tissue susceptibility to each cytokine. Severe malaria has long been linked to elevated levels of inflammatory cytokines including tumor necrosis factor (TNF-a), IL-1, and IL-6 in the blood. (10) Anti-inflammatory cytokines have since been discovered to play critical roles in the immune response to Plasmodium. IL-10 acts as an immunoregulator during

Plasmodium falciparum infection, neutralizing the influence of other cytokines released by Th-1 and CD8 cells.(11)

Interaction of plasmodia with the immune system

There is widespread consensus that both cell-mediated and antibody-dependent immunity are needed for adequate defense with different mechanisms possibly fine-tuned over time . .(12)

innate immunity is believed to play an important role in the removal of Plasmodium from parasitized hosts. In natural conditions, the spleen eliminates the majority of the waste, although the liver has been found to serve as an additional clearance site. (13)

These findings are consistent with evidence linking splenic/T lymphocytes and natural killer (NK) cells to the early development of gamma interferon (IFN-) and tumor necrosis factor alpha (TNF-).(14)

As immunity to malaria fades rapidly, another effect of decreased immune defense is decreased susceptibility to the infection and, as a result, a lack of frequent immune boosting. As a result, the malaria burden is expected to rise until newer preventive and treatment methods are developed. Gaining mechanistic insight into the processes involved will be one path toward this objective.(15)

The capacity of P. falciparum infected red blood cells to sequester in the deep endothelia of essential organs such as the brain, liver, and spleen results in the aggregation of toxic parasite components at sites of sequestration, resulting in a heavy induction of proinflammatory cytokine formation, endothelial destruction, organ dysfunction, and lifethreatening pathological disease. (16)

Several studies have shown that parasite glycosylphosphatidylinositols (GPIs) are one of the parasite toxic factors that contribute to malaria pathogenesis (17)

This hypothesis was based on GPIs' ability to trigger the development of TNF, IL1, IL6, and IFN in macrophages and cause symptoms similar to extreme malaria (SM) illnesses, such as pyrexia, hypoglycemia, and lethality.(10)

The theory was supported further by the fact that immunization with parasite GPIs decreased inflammation associated with acute Plasmodium berghei infection in mice 13, and that GPIs stimulate CD36, TLR2 and TLR4dependent signaling cascades to induce inflammatory cytokines and nitric oxide release from human macrophages in vitro .(18)

The association between GPIinduced inflammatory cytokines, such as TNF, IL6, and IL1, and IgG responses and outcome in SM in humans, is not well known. We previously recorded that low antiGPI IgG responses correlate with cerebral malaria (CM) cases in urban hypoendemic areas, while relatively high levels of antiGPI antibodies correlate with mild malaria (MM) (19)

Role of innate immune response against (malaria)

In addition to Pf-infected erythrocytes, natural killer (NK) cells are among the first cells in the peripheral blood to express IFN-gamma (Pf-E). NK cells can be present in the blood, secondary lymphoid glands, and peripheral non-lymphoid tissues.(20)

They take part in host innate responses to viral and intracytoplasmic bacterial infections, as well as tumor growth and allogeneic transplantation. This lymphocytes not only play a significant role in endogenous effector responses, but they also play a role in the initiation and production of adaptive immune responses.(20)

Furthermore, direct sensing of Pf infection by NK cells causes them to produce the proinflammatory chemokine IL-8, implying that NK cells play a role in the recruitment and activation of other cells during malaria infection.(21)

In the innate immune response to Pf, some other cell subsets are involved. The presence of the parasite can be detected by dendritic cells, macrophages, gamma delta T cells, and NKT cells. Along these lines, the presence of IL-12 is required for NK cell IFN-gamma formation, and functional cooperation occurs between macrophages and NK cells during this parasitic infection.(21)

This NK response is aided significantly by IL-18 provided by macrophages. However, the molecular basis of Pf-E identification by NK cells, as well as the functional involvement of NK cell responses during disease progression, remain unresolved.(21)

Role of cell mediated immunity

Cell-mediated responses to P. falciparum tend to be higher in HbAS individuals than in HbAA individuals. The mean lymphoproliferative response to affinity-purified P. falciparum soluble antigens was shown to be slightly higher in HbAS children relative to HbAA children but a substantial gap between HbAA and HbAS adults has not been reliably found .(22)

As a result, the available data point to a more robust cellular response to P. falciparum in HbAS infants. However, it is unknown if this is a result of HbAS's defensive effects. In HbAA individuals, the lymphoproliferative response is blocked during and after acute malarial infection .As a result, individuals with HbAS can have a more vigorous lymphoproliferative response. As a result, a stronger lymphoproliferative response in HbAS individuals could be secondary to other pathways of malaria defense.(22)

Role of humeral immunity

Researchers discovered signs of an increased humoral response in HbAS patients. Gamma globulin levels were shown to be higher in HbAS children relative to HbAA children .(23)

In most studies, higher levels of particular antibodies directed at parasite surface antigens thought to play a role in defensive responses, such as Pf155/ring infected erythrocyte surface antigen (RESA), merozoite surface protein 1 (MSP1), merozoite surface protein 2 (MSP2), erythrocyte binding antigen 175 (EBA175), and glutamate-rich protein (GLURP), were not seen in HbAS individuals compared.(24)

One study discovered higher levels of antibodies against free parasite antigens apical membrane antigen 1 (AMA1), EBA175, MSP1, MSP2, MSP3, circumsporozoite protein (CSP), and parasite schizont extract (PSE) in HbAS vs HbAA children living in low malaria transmission areas .(24)

Individuals with HbAS, on the other hand, have higher amounts of IgG aimed at PfEMP-1 family proteins found on the surface of red blood cells, according to a number of studies. According to one study, HbAS patients had a higher IgG response to tainted red blood cells in vitro. The Gambia , Gabon , and a low transmitting region of Burkina Faso are all affected. (25)

HbAS children had higher levels of IgG antibodies to PfEMP-1 than HbAA children . However, experiments in high malaria transmission areas failed to find improved antibody response to PfEMP-1 in HbAS children , perhaps because robust responses were seen in the majority of children in these high malaria transmission areas. Other reasons for differences amongst these. Methods of antigen processing and sample size limits are two other reasons for inconsistencies between these experiments.(26)

The discovery of elevated levels of IgG against PfEMP-1 but not against other parasite antigens indicates that the improved humoral immune response in HbAS

patients could be directed at proteins on the surface of the infected red blood cell. This phenomenon may be explained by increased splenic absorption of compromised red blood cells in HbAS patients, resulting in better appearance of surface antigens. (27)

Furthermore, defense against high parasite densities observed at younger ages can boost the development of acquired immunity, as parasitaemia may interfere with the development of successful immune memory .(27)

Higher levels of PfEMP-1 antibodies can provide defense in HbAS patients by enhancing opsonization and phagocytosis of infected red blood cells or by destabilizing cytoadherence. Accelerated antibody acquisition to PfEMP-1 may thus explain the age-dependent rise in HbAS defensive effects observed in three studies (28)

inflammatory and regulatory cytokine responses to malaria in children

Cytokines and chemokines are elevated in peripheral blood during an acute malaria outbreak and contribute to parasite elimination, but they are also likely to be responsible for much of the signs and pathological modifications observed during malaria disease. Experiment results show that the combination between pro and anti-inflammatory signals plays a significant role in determining the outcome of an infection, (29)

According to experimental evidence, the combination of pro and anti-inflammatory signals plays a significant role in determining the outcome of an infection, i.e. whether it contributes to defense and/or immunopathology . As a result, recent data from our community and others show that therapeutic immunity could also be dependent on the ability to control pro-inflammatory responses.(30)

Indeed, based on their plasma cytokine profiles, malaria-naive adults experimentally infected with P. falciparum can be classified into two prognostic classes (31)

1. The first group has a strong inflammatory response (defined as eight to 82-fold increases in IFN above baseline, with equally substantial increases in TNF, IL12p70, and the chemokine MIG/CXCL9) and has fast parasite control but worse symptoms.
2. The second group shows poor parasite management and less symptoms due to early TGF activity and a lower inflammatory response (less than a twofold rise in IFN over baseline).

According to the findings, younger children, like naive and partly immune adults, are more likely to mount a robust pro-inflammatory response to malaria, but this response is offset by significant quantities of regulatory cytokine release. Older children's responses were comparatively muted, implying age-related variations in immunity and the production of partial therapeutic resistance.(32)

Cytokines in the immunopathology of malaria

Malaria pathogenesis is complicated, as it is most certainly a combination of immunologic and nonimmunologic pathways. It is now well recognized that extreme malaria is caused by variations in a variety of tissues and organs. These flaws often cause metabolic acidosis and localized ischemia.(5)

This segment focuses on the role of cytokines in immunologic pathways. It is clear that parasite factors may contribute to disease severity, as shown by their ability to infect a high percentage of erythrocytes or cause the development of proinflammatory cytokines. (33)

It is worth noting that recent research indicates that the existence of anti-glycosylphosphatidylinositol antibodies in patients' serum can provide defense

against clinical malaria symptoms . As a result, cytokines, as possible pathogenic factors, may contribute directly or indirectly to a variety of pathological processes (34)

Experiments with IFN-/ or IFN-R/ have consistently shown that this cytokine is needed for the growth of CM. Overall, TNF- production and ICAM-1 expression or IL-12 production were inhibited in these animals' experiments. Surprisingly, NO does not seem to be involved in the pathogenesis of CM .(35)

Furthermore, IRF-1/ mice infected with P. berghei are immune to CM , indicating that IL-12 and IFN- play an important pathological role. Surprisingly, sex-related determinants can play a role in deciding IL-10's relative importance. TNF- seems to play an important role in CM.(36)

A potential cause may be found in the timing and location of cytokine synthesis. TNF-, which is developed locally in the central nervous system, has been found to play an important role in the production of CM .(36)

An significant question is whether data on human CM are consistent with the picture emerging from mouse studies. According to some studies, histopathological features observed in human CM are correlated with local development of TNF-, IFN-, and IL-1, as well as IL-10 (32)

A monkey variant of CM yielded similar findings . Other researchers, however, have discovered that the mRNA of proinflammatory (TNF- and IL-1) cytokines identified in postmortem samples of CM patients does not correspond with the density of parasitized erythrocytes . Patients infected with neutralizing anti-TNF- antibodies, on the other hand, provide a quicker resolution of clinical symptoms.(37)

Furthermore, patients with CM had a focal aggregation of TGF-1, -2, and -3 during brain parenchyma reorganization, indicating endothelial activation and

immunologic dysfunction .Interestingly, proof has been gathered in both of those cases that shows that the cause for this relationship is an increased capacity to generate TNF .(38)

Anemia is a condition of which there is a lack Anemia is one of the most puzzling of the physiopathological mechanisms underlying extreme malaria. Many theories have been suggested to explain how erythrocyte integrity is affected. None of them, though, offer a satisfactory justification for the reported magnitude. It has been proposed that host-related causes are the primary cause of malarial anemia.(39)

In vivo neutralizing experiments have shown that IFN-, TNF-, and IL-1 are not the primary cytokines involved in the inhibition of erythropoiesis . Other hematological modifications do not include changes in stem cell factor or IL-3 levels .Anemia has been linked to elevated IL-3 levels and an increase of IL-3-responsive, IL-4-producing non-B, non-T cells in people afflicted with blood-stage P. chabaudi . So far, the majority of the evidence suggesting an immunological intervention in malarial anemia has come from studies on the role of IL-12 in this pathology. (40)

Anemia is associated with high levels of IL-12, a cytokine that promotes erythropoiesis . Human studies have also shown the significance of immune systems in the incidence of extreme anemia in malaria. The level of anemia is proportional to the amount of neopterin in the blood discovered a connection between the highest levels of TNF- in serum and extreme anemia. (40)

This, though, is not a universal observation. Low levels of this cytokine were found in the serum of ghanaian children with SA in another study . This disparity may be interpreted by previous research showing that the ratio of IL-10 to TNF- in serum is a more reliable predictor of anemia severity.(41)

Patients with extreme anemia had low levels of IL-10, which were inadequate to combat the proinflammatory action of elevated TNF- levels. Other research backs up these conclusions. Low IL-10/TNF- ratios are associated with extreme anemia, implying that IL-10 can play a role in preventing TNF-'s detrimental effects on hematopoiesis. (29)

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