MOLECULAR AND IMMUNOLOGICAL ASPECTS OF ANEMIA IN MALARIA

Sri-Hidajati BS

ABSTRACTS

Anemia is an inevitable consequence of malaria especially in children and severe anemia becomes one of the complications in falciparum malaria. Anemia and malaria, both become serious problems in developing countries. For this reason a literature study was done to know the mechanisms and pathogenesis of anemia in malaria. Plasmodia live in red blood cells, consume and use hemoglobin for their growth and replication and at the end schizonts rupture and destroy their erythrocyte host cells. Those mechanisms bring us to the assumption that high parasitemia will cause higher degree of anemia. But severe anemia can be found also in children with low parasitemia and anemia can persist for weeks after parasite clearance by anti-malaria treatment. Literature study revealed that many other factors also play role in the development of anemia in malaria. First, infected erythrocytes with changes in surface property and lost deformability will be easily recognized and cleared up in the spleen. The presence of antibody and immune complex on the surface of erythrocytes makes them good targets for ADCC, complement-mediated lysis and erythrophagocytosis. This could involve non-infected erythrocytes, too. Macrophages and cytokines, especially TNF-α, IFN-γ and IL-1 have important role in malaria anemia. These cytokines enhance erythrocyte destruction, increase splenic clearance capacity, enhance erythrophagocytosis and depress bone marrow capacity for erythropoiesis. Cytokines also contribute in the increased uptake and intracellular storage of iron by macrophages, where iron is used for production and functioning of ROI and NO. Iron uptake and use in macrophages causes iron decrease in the circulation, and this causes decrease of parasite growth but also impairs erythrocyte production in bone marrow. Other cytokines, IL-10 and TGF-β represent counter response against the effects of TNF-α and IFN-γ. IL-10 was proved to stimulate bone marrow function in vitro. IL-10 concentration is higher in malaria-resistant mice and TGF-β is found in resolving malaria infection. The reviewed reports could explain the mechanisms of anemia in malaria, but no conclusive reports of prevention and treatment suggested against the anemia related to the mechanisms mentioned. There were some reports about the effects of anti-malaria drugs on TNF, RNI and ROI production capacity, but still no practical use suggested.

Keywords: *malaria, anemia, cytokines, antibody*

INTRODUCTION

Anemia is a consequence of malaria especially in children. In falciparum malaria, anemia can be severe that hemoglobin concentration can be lower than 5 gram percent (Pasvol, et al., 1995). Anemia and malaria, both become problems in developing countries where poverty, under nutrition and infectious diseases constitute a vitiate circle difficult to cope. With the findings reported by Murray et al. in Nigeria that in malnourished individuals, a change of diet from famine to a nearly normal may precipitate attacks in patients with existing quiescent falciparum malaria infection (Murray , et al., 1975), the problem becomes more complex. From the parasitologic point of view, it is obviously known that rupture of mature schizonts into merozoites destroys the erythrocyte host cells.

Department of Parasitology
Airlangga University School of Medicine
Tropical Disease Center, Airlangga University,
Surabaya

High parasite rate is thought to result in higher rate of anemia. But evidence showed that severe anemia can be found in children with low parasite rate and anemia can persist for weeks after parasite clearance by anti-malaria treatment (Melancon-Kaplan, et al., 1993). Those facts raise questions about the mechanism of anemia occurs in malaria patients. Literature search revealed numerous possible mechanisms of anemia in malaria as will be reviewed below.

ANEMIA IS RELATED TO PARASITE GROWTH AND REPLICATION

Schizonts rupture at the end of asexual erythrocytic cycles destroys the host cells. Plasmodia take new erythrocyte hosts every 36 to 72 hours dependent on the species. This logically contributes to the occurrence of anemia in malaria. Plasmodium falciparum, which has relatively short cycle and produces numerous descendants per cycle, destroys more erythrocytes then other species (Cheesbrough, 1987).

Malaria parasites replicate quickly within the erythrocytes. One invading merozoite produces 8 to 40 daughter parasites per cycle, depending on the species (Howard, 1988). The rapid replication rate calls the need for big amount of nutritional stuffs not only for their growth and reproduction, but also for maintenance of cellular structures and functions. Malaria parasites have limited ability to synthesize their own amino acids, therefore they import most of the amino acids from their environment. Hemoglobin in the erythrocyte cytosol provides most of the amino acid requirement, while only few amino acids are taken from the outside of erythrocytes (Howard, 1988; Rosenthal and Meshnick, 1998). While hemoglobin is an easy-to-find source of protein in the erythrocytes as it constitutes 95% of total erythrocytic protein (Anonym, 2004).

Parasites take up erythrocytic cytoplasm by pinocytosis or via special feeding structure like cytostomes, and transport the stuff inside double-membrane vesicles that later fuse into/with the food vacuoles where hemoglobin is degraded and the components are separated. The globin portion is hydrolyzed into small peptides, which are sent back to the parasite cytoplasm where the final conversion into amino acids proceeds. The liberated heme portion (free heme) is toxic due to its capability to inhibit the activity of several enzymes and to destabilize and lyse membranes. To neutralize the toxicity, several methods are employed by the parasites; one of them is conversion or bio-crystallization into macromolecule called hemozoin, b-hematin or malarial pigment.

The metabolic process of hemoglobin especially the steps proceed in the food vacuoles, becomes the target of many antimalarial drugs, e.g., Chloroquine, Amodiaquine and other aminoquinoline derivatives which inhibit hemoglobin degradation process and hinder pigment formation, and especially let the toxic free heme unneutralized (Krogstad and De, 1998; Rosenthal and Meshnick, 1998; Anonym, 2004).

As the result of degradation and consumption by the parasites, hemoglobin content of infected red blood cells decreases around 25-75 percent during the parasite life cycle. As calculated, at 20% parasitemia 110 g hemoglobin was consumed during 48 hours (Rosenthal and Meshnick, 1998; Anonym, 2004). Hemoglobin may also provide iron for the parasites beside some other sources of iron, i.e. erythrocyte free iron, erythrocyte ferritin, and plasma free or transferrin-bound iron (Howard, 1985; Rosenthal and Meshnick, 1998; Anonym, 2004).
That iron is needed for the growth of malaria parasites was confirmed by the findings of Murray et al. from a famine area in Eastern Nigeria in 1975, that in malnourished patients, a change of diet from famine to a nearly normal diet may precipitate attacks in patients with existing quiescent falciparum malaria infection. Laboratory examination showed that serum iron and transferrin saturation increased quickly after re-feeding and peaked at 48 hours but was followed by the increase of parasitemia. The level of parasitemia, with starting point of 2% on admission day, peaked to around 15% at five day, when serum iron concentration has been decreased. To confirm the phenomenon, they did experiments in rats and they found that in rats receiving iron injection (with resulting transferrin saturation value 99%), parasitemia rose more quickly, reached higher levels and the rats died earlier than the control group receiving placebo which showed transferrin saturation 43% (Murray et al., 1975).

INFECTED ERYTHROCYTES ARE PRONED TO SPLEEN CLEARANCE MECHANISM

Normally, aged red blood cells are destructed by a mechanism in the spleen and damaged erythrocytes are then phagocytosed by macrophages. The spleen has the role for surveillance and removal of cells with abnormal mechanism or surface property. Erythrocytes with reduced deformability are removed as are erythrocytes containing inclusions. Young and normal erythrocytes have sufficient deformability that they can slip well through the small blood vessels of the spleen. In malaria patients, infected erythrocytes become distended and lose their deformability and their capability to slip through the small fenestrations in the endothelial cell wall. They are easier trapped within vascular network in the spleen and become good targets for the clearance mechanism. Intracellular malaria parasites, especially the old ones, represent large inclusions to be the target of removal system. The spleen also has immune mechanism to recognize cell surface changes, specific or non specific like changes in ionic charge or carbohydrate contents while malaria parasites have the need and capability to export their products to, or/and change the surface property of their erythrocyte host membrane (Howard, 1988; Miller, et al., 1989). Higher concentration of oxygen radicals leads to premature aging of erythrocyte, and this is valid not only to the infected erythrocyte but also to the uninfected ones. (Melancon-Kaplan, et al., 1993). Against the clearance mechanism of the spleen, Plasmodium falciparum develops later an evading mechanism by cyto-adherence and sequestration in other organs (Berendt, Fergusson and Newbold, 1990).

THE ROLE OF ANTIBODY IN MALARIAL ANEMIA

Antibody response play role in malarial anemia. As already discussed before, malaria parasites inside the erythrocytes express antigen on the surface of its host red blood cells. Parasite-specific antibodies, i.e. IgM, IgG1, IgG2, IgG3, IgG4 can be found in the sera of people from endemic areas (Howard, 1988; Deloron, et al., 1990; Sarthou, et al., 1997). Antibody response can call out the occurrence of ADCC (antibody dependent cellular cytotoxicity) and complement mediated hemolysis. The presence of autoantibody against red blood cells was also reported in malaria endemic areas. Malarial IgG was found on the membrane of infected and uninfected erythrocytes that can activate classical complement system leading to erythrocyte lyses. Most of malaria infected patients show positive direct Coomb test (Melancon-Kaplan, et al., 1993).

The presence of antigen-antibody complex on the erythrocyte surface also leads to phagocytosis and opsonization. The complexed antibody attach to the phagocytic cells like macrophages and neutrophils as ligands for specific receptors (FcgR and FceR) on the surface of phagocytes through the Fc region (Howard, 1988; Roitt, 2002). In malaria-infected individuals, immune serum containing antibody, complement, and cytokines like IFN-γ and TNF-α activate macrophages. Activated macrophages and neutrophils increase their phagocytic capacity and release substances for erythrocyte destruction (Kremsner, et al., 1989; Kumaratilake, et al., 1992).

THE ROLE OF CYTOKINES AND MACROPHAGE IN MALARIAL ANEMIA

Clark (1987a, 1987b; 1988a, 1988b) reported that destruction of erythrocytes in malaria-infected persons was mediated by reactive oxygen intermediates released by macrophages and neutrophils. The release of ROI and the increased capacity of phagocytosis is attributable to the effects of TNF-α and related cytokines. As mentioned above, the rate of splenic destruction was increased in malaria, and TNF was found in immuno-histochemistry of the malarial spleen. TNF-α and some other cytokines are produced and released in response to malaria infection and its overproduction is implicated to the pathogenesis of malaria disease and severity. Strains of Plasmodium falciparum vary in their ability to stimulate TNF-α production (Clark 1981; Allan, et al., 1995). Clark also reviewed that the rate of erythrophagocytosis is higher in malarial patients. Splenic macrophages and Kupffer cells in the liver were seen to take up sequestered erythrocytes (Weiss, 1995).

The production of red blood cells is also impaired in malaria. According to Broxmeyer, TNF is one of cytokines which can depress bone marrow for erythrocyte production. Evidence of dyserythropoiesis lead by TNF-α was confirmed by the following investigation: injection of TNF-α in early stage of malaria in mice can lead to dyserythro-poiesis and also increased erythrophagocytosis. Dyserythropoiesis and increased erythro-phagocytosis could be reversed by administration of neutralizing antibody against TNF-α, but not by administration of erythropoietin (Broxmeyer, 1995). To investigate which steps of erythropoiesis are impaired by TNF-α, Miller (Miller, et al., 1995) did experiments with mice and found that injection of murine TNF-α could reduce the number of pluripotent stem cells in the bone marrow while they increased the number in the spleen. TNF-α injection also reduced the number of erythroid progenitor cells and reduced incorporation of radioactive-labeled ferrum into erythrocytes. If the malaria-infected mice were injected with antiserum against recombinant TNF-α, erythropoiesis would be partially restored. Stem cell depletion in the bone marrow happened before anemia was detectable in the animal. Miller also reviewed other findings that TNF in malaria can play role in enhancing the differentiation of pluripotent stem cells into myeloid progenitors in the expense of erythroid development (competitive among stem cell lineages), and TNF produced by monocytes also act as autocrine to induce monocyte replication by releasing M-CSF (macrophage-colony stimulating factor). According to Miller the role of cytokines in anemia in malaria is performed directly by inhibiting erythroid precursors and indirectly by stimulating the release of tissue damaging radical substances, enhancing monocyte function in erythrophagocytosis and promoting monocyte differentiation at the expense of erythrocyte development.

According to Rusten, TNF-α shows both stimulatory and inhibitory effects on proliferation of hematopoietic progenitor cells. Those effects are mediated directly or indirectly by stimulating accessory cells to cytokine production. TNF-α inhibits the growth of high proliferative potential colony-forming cells (HPP-CFCs), the most primitive human bone marrow progenitor cells, which is naturally stimulated by multiple growth factors, through the involvement of both TNF receptors, p55 and p75. Low, but not high concentration of TNF-α could stimulate IL-3- and GM-CSF- induced HPP-CFC colony formation. This is mediated only by p55 (Rusten, et al., 1994). While other acute phase protein, i.e. a1-antitrypsin and a2 -

macroglobulin may also cause ineffective erythropoiesis by blocking transferrin-mediated uptake of iron into reticulocytes and erythroid progenitor cells (reviewed by Weiss, et al, 1995 and Schlichterle, et al., 1996). Taverne and her group later completed the evidence with experimental results that transgenic mice with TNF-α overproduction suffered from severe anemia (Taverne, et al., 1994).

IL-1, according to Beutler and Cerami (1990) did not have any suppressive effect on bone marrow while Weiss reviewed some reduction effects of IL-1 on proliferation of erythroid progenitor cells (Weiss, et al., 1995). IFN-γ is also one of central suppressors of erythroid colony formation. IFN-γ interfere the result of erothropoietin treatment trials for anemic patients and made it "blunt" and doubtful (Broxmeyer, 1995; Weiss, et al., 1995).

Macrophage needs iron for its work to destroy invading microorganisms by its cytotoxic capacity. Iron contributes in the production of several reactive oxygen species. In relation with that work, activated macrophages have a central role in the pathogenesis of "anemia in chronic diseases" (ACD) by inducing hypoferraemia as the result of increased uptake of iron and incorporation of the iron into intracellular storage form as ferritin. When at last macrophages are concentrated in the reticulo-endothelial system, iron is decreased from the circulation. This would in fact give negative impacts for the growth of the invading microorganisms including malaria parasites. On the other hand, withdrawal of iron from bone marrow and erythroid progenitor cells also results in reduction of haem synthesis and global oxygen transport capacity (Weiss, et al., 1995). Activation of macrophages in ACD is shown by the increase of Neopterin concentration. Neopterin is produced in excess by macrophage upon stimulation with IFN-γ. Its concentration in serum has been used as a clinical marker of the activation of cellular mediated immune response in vivo. Other indicators of macrophage activation is the serum level of IFN-γ itself and soluble 75 kD TNFR (one of two receptors for TNF-α which is released from the cell surface to the circulation). Neopterin, IFN-γ and TNFR concentrations show inverse correlations with serum iron concentration and hemoglobin levels and positive correlation with ferritin concentrations in many diseases (Kremsner, et al., 1989; Weiss, et al., 1995). IFN-γ and IL-2, enhances strongly the expression of transferrin receptors, the essential protein for iron uptake to the monocytes. While TNF-α and IL-1 induce synthesis of H-chain ferritin, protein needed for the intracellular storage of the acquired iron. This constitutes a part of acute phase response induced by IL-1 and TNF-α. IL-6 also participates in the induction of iron uptake and ferritin synthesis in hepatocytes. The impact of increased uptake and incorporation of iron into ferritin by macrophages may cause growth limitation of invading microorganism by iron restriction as mentioned before and by enhancement of the activity of cytokines like IFN-γ and TNF-α. Both cytokines have killing effects on micro-organisms. On the other hand, iron loading can reduce the effect of IFN-γ, whereas iron starvation following treatment with iron chelator desferrioxamine (one of the candidate for anti-malaria drug) causes an increase in IFN-γ activity. Similarly for TNF-α, iron loading on macrophages will reduce its production and activity, while administration of iron chelator will upregulate the expression of TNFRs on the surface of macrophages. Activated macrophages are also capable of synthesizing transferrin which works as paracrine to promote lymphocyte proliferation to overcome the depression effect of hypoferraemia on the immune system (Weiss, et al., 1995).

NO (nitric oxide) is a central component of macrophage-mediated cytotoxicity in mammals. NO has been proved to take part in the killing of malaria parasites (Kremsner, etal., 1993). NO is produced from L-Arginine under catalysis of NO-synthase (NOS), a haem-containing enzyme. Besides macrophages, NOS is also found in endothelial lining of blood vessels, nervous system and many other tissues including liver. There are two kinds of NOS, i.e. constitutive NOS (cNOS) which mediates production of NO needed for physiological processes, and (cytokine-)inducible NOS (iNOS) which works on response to non-physiologic inducer, e.g. infection. NO production and activity in infection like malaria was reported to be incited by microbial antigen, cytokines like IFN-γ, TNF-α and IL-1 (Moncada, Palmer and Higgs, 1991; Lancaster, 1992; Weiss, et al., 1995). Biological effects attributable to NO (i.e.toxic effects) are mediated through its interaction with iron-containing enzymes. The cytotoxic effects of NO can be seen as repression on DNA synthesis, disturbance of mitochondrial respiration, and interference on Krebs cycle in target cells (Weiss, et al., 1995). IFN-γ also amplifies TNF-α production, whereas TNF-α in autocrine way increases its own production. Increased TNF-α concentration results in higher NO synthesis (Farrar and Schreiber, 1993; Roitt, 2002). Malaria parasite is capable to stimulate production of NO and high level of NOS activity is found in lysate of infected erythrocytes (Ghigo, et al., 1995). NO also modulate intracellular iron regulation. The modulation is exerted in post- transcriptional step via IREs (iron responsive elements). A high intracellular concentration of low molecular weight non-ferritin-bound iron inactivates IRE-binding activity of IRP (iron regulatory protein) that induces degradation of transferrin receptor

mRNA and increases translation of ferritin and e-ALAS (= erythroid-5-aminolaevulinic acid synthase, enzyme for haem biosynthesis) mRNA leading to an decrease uptake of iron into the cell and increases iron storage process in the form of ferritin-bound and increases haem biosynthesis, too. On the opposite, when iron deprivation occurs, IRE -binding activity of IRP is stimulated leading to stabilization of transferrin receptor mRNA and repression of ferritin and e-ALAS mRNA translation, that causes enhancement of iron uptake and reduction of iron storage and haem biosynthesis. NO has the capability to activate IRE-binding activity of IRP, and regulatory effects of NO are reflected by alterations in ferritin synthesis and expression of transferrin receptor, in the same way with iron-induced regulation of IRP. On the other hand, the expression of iNOS is regulated by the concentration of intracellular iron. Increased concentration of intracellular low molecular weight iron reduces IFN-γ-mediated transcription of iNOS and consecutive formation of NO while deprivation of intracellular low molecular weight iron enhances expression of iNOS and production of NO. This autoregulatory system may be responsible for the altered iron traffic in ACD.

The increased uptake and storage of iron by macrophages according to this model brings two consequences: 1. it would result in the limitation of iron availability for the growth of invading microorganisms; 2. it would enhance the cytotoxic potential of macrophages with the increasing production of NO, the main effector molecule of antimicrobial toxicity in macrophages (Weiss, et al., 1995). Murray's report cited on previous paragraph shows more evidence that increase in serum iron in ACD leads to increased growth of malaria parasites (Murray et al., 1975).

IL-10 and TGF-β (transforming growth factor-beta), two anti-inflammatory cytokines have been reported could down-regulate the production of TNF and IFN-γ (Abbas, 1998). The level of circulating TGF-β and production of bioactive TGF-β by splenocytes are low in lethal dose injection of Plasmodium berghei in mice, but a resolving infection was accompanied by significant increase in TGF-β production. Treatment of P. berghei-infected mice with recombinant TGF-β slowed the rate of parasite proliferation, significantly decreased serum TNF-α level and increased IL-10. Treatment of infected mice with neutralizing antibody against TGF-β exacerbates the virulence of Plasmodium infection and transforms a resolving infection into lethal condition (Omer and Riley, 1998).

Investigations revealed that concentration of IL-10 mRNA in the spleen and the brain of malaria-resistent animal models is higher than the susceptible animals, and neutralization antibodies against IL-10 could induce the appearance of cerebral malaria (Kossodo, et al., 1997). The addition of exogenous IL-10 could reduce mRNA accumulation of TNF-α, IL-1 b and IL-6. It was thought that severe malaria occurs in the case where no adequate feedback from IL-10 proceeds (Ho et al., 1998). The phenomenon is noted in severe anemia as well (Kurzhals, et al.,1998). Omer and Riley (1998) also found that IL-10 stimulates bone marrow function in vitro and counteracts anemia in mice. Kurzhals in the study among 175 African children with malaria, found that IL-10 concentrations were significantly lower in patients with severe anemia than in uncomplicated malaria and cerebral malaria. From this study, he also concluded that severe anemia in malaria occurs when no sufficient (IL-10) response takes place to a high TNF concentrations (Kurzhals, et al.,1998). Ching Li proved further that IL-10 deficient mice suffer more severe disease when infected with P. chabaudi and the pathology of malaria in this animal can be decreased by the administration of anti- TNF-α (Li, et al., 2003). Nussenblatt et al. (2001) found that the concentrations of TNF, erythropoietin and IL-10 were significantly higher in younger children (aged 12-24 months) suffered from malaria than older ones. This can explain why anemia related to malaria is more prevalent in younger children in malaria endemic areas.

RESEARCH DONE TO COPE WITH FACTORS WORK IN MALARIA ANEMIA

As mentioned on the first passages, the presence and number of parasites takes role in the degree of anemia. While strain of parasites influences the capability of parasites to stimulate TNF-α production, an important factor in the occurrence of anemia in malaria. Treatment of patients with anti-malaria drugs, as far as no resistance exists, could reduce the level of parasitemia, followed by decrease of TNF concentration. Anti-malaria drugs also act directly to inhibit hemoglobin degradation and further metabolism by the parasites, but the killing effects are more important. The recovery of patients from malaria can be followed by the increase in hemoglobin concentration to normal level (Sri-Hidajati, 2001; 2006 in press).

In cases where resistance to antimalarials occurs, contradictory findings have been reported before. From a study among malaria cases in Flores, Hoffman noted that the use of Chloroquine, as therapeutic or prophylactic drug, despite parasite resistance, could prevent patients from suffering severe disease and death (Hoffman, et al., 1984). Picot et al. also reported similar finding from a field study in the Gambia and confirmation studies have been done in the laboratory

that most anti-malaria drugs have inhibitory effects on TNF production (Picot et al.,1991; 1997; Kwiatkowski and Bate,1995). Chloroquine was also reported to have inhibitory effects on other functions of monocytes, e.g. phagocytosis (Osorio, 1992) and NO production (Kremsner, et al.,1993a). Many other reports were published about the potential inhibitory effects of Chloroquine on the immune response (Byorkman, 1988; Gyhrs, et al., 1991; Freyauff, et al ., 1997). Whereas analysis on TNF concentrations in Chloroquine-treated patients failed to show direct inverse correlation of TNF levels and Chloroquine, that could mean there was a time lapse before TNF decreased after Chloroquine treatment (Sri-Hidajati, 2001). Furthermore, Bate et al. detected the presence of a T-independent, transient IgM antibody in acute malaria patients which could prevent or inhibit TNF-α production (Bate, et al., 1990; Playfair, et al., 1990). As already mentioned above, dyserythropoiesis and increased erythrophagocytosis in experimental malaria in mice, could be reversed by administration of neutralizing antibody against TNF-α, but not by administration of erythropoietin (Broxmeyer, 1995). The use of antibody against TNF-α was reported in Gambian children where the antibody showed effects in term of reduction of fever, but showed no reduction on fatality rate (Kwiatkowski, et al., 1993). Pentoxifylline, was proved experimentally to reduce steady-state TNF-α mRNA but had no effect on TNF-α mRNA translation in malaria (Grau and Behr, 1995). Clinically, it has been demonstrated to inhibit TNF-α production in higher dosage, but it showed enhancement effects at low concentrations (Jakobsen, Koch and Bent, 1997). In vivo study of corticosteroids done by Moncada and his groups showed that they could inhibit the production of TNF-α and inhibit the action of iNOS (Moncada, Palmer and Higgs, 1991), but clinical study by Hoffman, et al., (1988) proved, that high dosage of corticosteroids did not show any benefit to severe malaria cases.

Concerning Nitric Oxide and other reactive nitrogen intermediates, Kremsner et al. (1993b) found that anti-malaria drug such as Chloroquine inhibited RNI production in a dose-dependent way, Quinine also cause significant reduction of RNI production in a concentration within therapeutic range, while high concentration of Artelinate significantly inhibited IFN-γ induced RNI production, but Clindamycin had no effect , and halofantrine significantly enhanced IFN-γ induced RNI production (Kremsner, et al.,1993a). The study revealed further that ROI production was unaffected by anti-malaria drugs (Kremsner, et al., 1993a). Anti-TNF mAb inhibits the IFN-γ induced RNI production. Pentoxifylline reduced malaria-antigen and IFN-γ induced RNI production, but did not reduce TNF-induced RNI production (Kremsner, et al., 1993b).

CONCLUSIONS

From the literature study reported above, a conclusion can be drawn that many factors play role in the development of anemia in malaria patients. First, hemoglobin is consumed by parasites and used for their growth and replication, while the erythrocyte hosts are destroyed at the end of every cycle. Infected erythrocytes are good target for splenic clearance. The presence of antibody and immune complex on the surface of erythrocytes causes infected and non-infected erythrocytes become good targets for ADCC, complement-mediated lysis and erythrophagocytosis.

The role of macrophages and cytokines especially TNF-α, IFN-γ and IL-1 in malaria anemia is important. These cytokines enhance erythrocyte destruction and increase splenic clearance capacity, enhance erythrophagocytosis by macrophages and depress bone marrow capacity for erythropoiesis. Cytokines also contribute in the increased uptake and intracellular storage of iron by macrophages, which is useful for production of radical oxygen and nitrogen intermediates for killing malaria parasites. When later macrophages accumulate in the RES, iron is further decreased from the circulation. This also causes decrease in the growth of parasites. Decreased iron concentration in the bone marrow also causes impairment of erythrocyte production.

Anti-inflammatory cytokines IL-10 and TGF-β represent counter response against the effects of TNF-α and IFN-γ. IL-10 was proved to stimulate bone marrow function in vitro. IL-10 concentration is higher in malaria-resistant mice and TGF-β is found in resolving malaria infection. It was thought that anemia and other symptoms of severe malaria occur when counter response of IL-10 is absent. Many reports have been published concerning the efforts to overcome the factors working in malarial anemia. Some anti-malaria drugs have been reported of having effects on the production of TNF and NO, but no conclusive results exists and no guideline for practical use suggested so far.

REFERENCES

Abbas AK, Lichtman AH, Pober JS, 1998. *Cellular and molecular immunology*. Philadelphia: WB Saunders Co.

Allan RJ, Beattie P, Bate C, Van Hensbroek MB, Morris-Jones S, Greenwood BM, Kwiatkowski D, 1995. Strain variation in Tumor Necrosis Factor induction by parasite from children with acute falciparum malaria. *Infect Immun* 63(4), pp. 1173-1175.

Anonym, 2004. Biochemistry of Plasmodium, In: *Malaria Update* (from Basic Science to Clinical Practice), CD ROM, published by Farmedia.id.net

Bate CAW, Taverne J, Dave A, Playfair DHL, 1990. Malaria exoantigens induce T-independent antibody that blocks their ability to induce TNF. *Immunol* 70, pp. 315-20.

Berendt AR, 1992. *Sequestration and its discontent: infected erythrocyte-endothelial cell interactions in Plasmodium falciparum malaria*. 54th Forum in Immunology, pp. 21-8.

Beutler B, Cerami A, 1990. Cachectin (Tumor Necrosis Factor) and Lymphotoxin as primary mediators of tissue catabolism, inflammation and shock. In : Cohen S, ed. *Lymphokines and the immune response*. Boca Raton, Florida: CRC Press, Inc., pp. 199-212.

Broxmeyer HE, 1995. Role of cytokines in hematopoiesis. In : Aggarwal BB, Puri RK, eds. *Human cytokines: their role in disease and therapy*. Massachusset USA: Blackwell Science, Inc, pp. 459-76.

Cheesbrough, M, 1987. *Medical Laboratory Manual for Tropical Countries*. Vol 1, Kent, UK: Butterworth & Co, Ltd., pp. 221-51

Clark IA, Virelizier J, Carswell EA, Wood PR, 1981. Possible importance of macrophage-derived mediators in acute malaria. *Infect Immun* 32(3), pp. 1058-66.

Clark IA, 1987a. Cell mediated immunity in protection and pathology of malaria. *Parasitol Today* 3(10), pp. 300-5.

Clark IA, 1987b. Monokines and lymphokines in malaria pathology. *Ann Trop Med Parasitol* 81(5), pp. 577-85.

Clark IA and Chaudry G, 1988a. The balance of useful and harmful effects of TNF, with special reference to malaria. *Ann Inst Pasteur Immunol* 139(3), pp. 305-6.

Clark IA and Chaudry G, 1988b. TNF may contribute to the anemia of malaria by causing dyserythropoiesis and erythrophagocytosis. *Br J Haematol* 70(1), pp. 99-103.

Clark IA, Chaudry G and Cowden WB, 1988c. *Interplay of reactive oxygen species and tumor necrosis factor in tissue injury. Oxyradicals in molecular biology and pathology*. Alan R Liss, Inc., pp. 53-60

Clark IA, Cowden WB, 1999. Why is the pathology of falciparum worse than that of vivax malaria? *Parasitol Today* 15(11), pp. 459-61.

Deloron P, Cot M, 1990. Antibodies to ring-infected erythrocyte surface antigen and circumsporozoite protein of Plasmodium falciparum in a rural community of Burkina Fasso. *Trans Roy Soc Med Hyg* 84, pp. 191-5.

Deloron P, Dumont N, Nyongabo T, Aubry P, Astagneau P, Ndarugirire F, Menetrier-Caux C, Burdin N, Brelivet JC, Peyron F, 1994. Immunologic and biochemical alteration in severe falciparum malaria: relation to neurological symptoms and outcome. *Clin Infect Dis* 19, pp. 480-5.

Dinarello CA, 1990. Interleukin-1 and its biological related cytokines. In: Cohen S, eds. *Lymphokines and the immune response*. Boca Raton Florida, CRC Press Ch 8, pp. 146-179.

Farrar M, Schreiber R, 1993. Killing of Plasmodium falciparum by cytokine activated effector cells (neutrophils and macrophages). *Annu Rev Immunol* 11, pp. 57-611.

Ghigo D, Todde R, Ginsburg H, Costamagna C, Gautret P, Bussolino F, Ullier D, Giribaldi G, Deharo E, Gabrielli G, et al, 1995. Erythrocyte stage of Plasmodium falciparum exhibit a high nitric oxide synthase (NOS) activity and release an NOS-inducing soluble factor. *J Exp Med* 182, pp. 677-88.

Grau GE and Behr C, 1995. Cytokines and malaria: for better or worse. In: Aggarwal BB, Puri RK, eds. *Human cytokines: their role in disease and therapy*. Massachusetts, USA : Blackwell Science, Inc, CH 30 p 459-76.

Ho M, Schollaardt T, Snape S, Looareesuwan S, Suntharasamai P, White NJ, 1998. Endogenous Interleukin-10 modulate proinflammatory response in Plasmodium falciparum malaria. *J Infect Dis* 178(2), pp. 520-5.

Hoffman SL, Rustama D, Punjabi NH, Sarumpaet B, Sanjaya B, Dimpudus AJ, McKee KT, Palcologo FP, Campbell JR, Marwoto H, Laughlin L, 1988. High-dose Dexamethasone in Quinine-treated patients with cerebral malaria: a double-blind, placebo-controlled trial. *J Inf Dis* 158(2), pp. 325-31.

Howard RJ, 1988. Plasmodium falciparum proteins at the host erythrocyte membrane: their biological and immunological significance and novel parasite organelles which deliver them to the cell surface. In: Englund PT, SherA, eds. *Biology of parasitism*. New York: Allan R. Liss Inc p 111-45.

Howard RJ, Handunnetti SM, Hasler T, Gilladoga A, Aguiar JC, Pasloske BL, Morehead K, Albrecht GR, Schravendijk MR, 1990. Surface molecules on Plasmodium falciparum-infected erythrocytes involved in adherence. *Am J Trop Med Hyg* 43(2) Suppl 15-29.

Kossodo S, Monso C, Juillard P, Velu T, Goldman M, Grau GE, 1997. Interleukin-10 modulates susceptibility in experimental cerebral malaria. *Immunol* 91(4), pp. 536-40.

Kremsner PG, Feldmeier H, Zotter GM, Jansen-rosseck R, Graninger W, Rocha RM, Bienzle U, 1989. Immunological alterations in uncomplicated Plasmodium falciparum malaria. Relationship between parasitemia and indicators of macrophage activation. *Acta Trop* 46, pp. 351-9.

Kremsner PG, Neifer S, Rasenac T, Bienzle U, 1993a. Interference by antimalarial drugs with the in-vitro production of reactive nitrogen intermediates by murine macrophages. *J Antimicrob Chemother* 31, pp. 385-92.

Kremsner PG, Nuessler A, Neifer S, Chaves MF, Bienzle U, Senaldi G, 1993b. Malaria antigen and cytokine-induced production of reactive nitrogen intermediates by murine macrophages: no relevance to the development of experimental cerebral malaria. *Immunol* 78, pp. 286-90.

Krogstad DJ and De D, 1998. Chloroquine : modes of action and resistance and the activity of Chloroquine Analogs. In: Sherman IW (editor). *Malaria: Parasite biology, pathogenesis and protections*. Washington : ASM Press Ch 23.

Kumaratilake LM, Ferrante A, Jaeger T, Rzepczyk M, 1992. Effects of cytokines, complement, and antibody on the neutrophil respiratory burst and phagocytic response to Plasmodium falciparum merozoites. *Inf Immun* 60(9), pp. 3731-8.

Kurtzhals JA, Adabayeri V, Goka BG, Akanmori BD, Oliver-Commey JO, Nkrumah FK, Behr C, Hviid L, 1998. Low plasma concentration of Interleukin-10 in severe malarial anemia compared with cerebral and uncomplicated malaria. *Lancet* 351(9118), pp. 1768-72.

Kwiatkowski D, Molineux ME, Stephens S, Curtis N, Klein N, Pointaire P, Smit M, Allan R, Brewster DR, Grau GE, 1993. Anti-TNF therapy inhibits fever in cerebral malaria. *Q J Med* 86(2), pp. 91-8.

Lancaster JR, 1992. Nitric oxide in cells. Amer Sci 80, pp. 248-59.

Li C, Sanni LA, Omer F, Riley E, Langhorne J, 2003. Pathology of Plasmodium chabaudi chabaudi infection and mortality in Interleukin-10-deficient mice are ameliorated by anti-Tumor Necrosis Factor α and exacerbated by anti-Transforming Growth Factor ? antibodies. *Infect Immun* 71(9), pp. 4850-6.

Melancon-Kaplan J, Burns JM, Vaidya AB, Webster HK, Weidanz WP, 1993. Malaria. In : Warren KS, ed. Immunology and Molecular Biology of Parasitic Infections, 3 ed. Blackwell Scientific Publ, Oxford etc, Ch 14 p302-51

Miller KL, Silverman PH, Kullgren B, Mahlmann LJ, 1989. Tumor Necrosis Factor α and the anemia associated with murine malaria. *Infect Immun* 57(5), pp. 1542-46.

Moncada S, Palmer RMJ, Higgs EA, 1991. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* 43(2), pp. 109-42.

Murray MJ, Murray NJ, Murray AB, Murray MB, 1975. Refeeding-malaria and hyperferraemia. *The Lancet* 653-4.

Nussenblatt V, Mukasa G, Metzger A, Ndeezi G, Garret E, Semba RD, 2001. Anemia and Interleukin-10, Tumor Necrosis Factor α, and Erythropoietin levels among children with acute, uncomplicated Plasmodium falciparum malaria. *Clin Diagn Lab Immunol* 8(6), pp. 1164-70.

Omer FM, Riley EM, 1998. Transforming growth factor beta is inversely correlated with severity of murine malaria infection. *J Exp Med* 188(1), pp. 39-48.

Pasvol G, Clough B, Carlsson J, Snounou G, 1995. The pathogenesis of severe falciparum malaria. *Bailliere's Clinical Infectious Diseases* 2 (2), pp. 249-70.

Playfair JHL, Taverne J, Bate CAW, de Souza, 1990. The malaria vaccine: anti-parasite or anti-disease? *Immunol Today* 11(1), pp. 25-7.

Riley EM, 1999. Is T-cell priming required for initiation of pathology in malaria infection? *Immunol Today* 20(5), pp. 228-33.

Roitt I, Brostoff J, Male D, 2002. *Immunology*, 6th ed. Ch 16 Immunity to protozoa and worms. Edinburgh : Mosby..

Rosenthal PJ, Meshnick SR, 1998. Hemoglobin processing and the metabolism of amino acids, heme and iron. In : Sherman IW (ed): *Malaria. Parasite biology, pathogenesis, and protection*. Washington : ASM Press,. Ch10 pp. 145-158.

Rudin W, Favre N, Bordmann G, Ryffel B, 1997. Interferon-gamma is essential for the development of cerebral malaria. *Eur J Immunol* 27(4), pp. 810-5.

Rusten LS, Jacobsen W, Lesslauer W, Loetscher H, Smeland EB, Jacobsen SEW, 1994. Bifunctional effects of Tumor Necrosis Factor Alpha (TNF-α) on the growth of mature and primitive human hematopoietic progenitor cells: involvement of p55 and p75 TNF receptors. *Blood* 83(11), pp. 3152-9.

Sarthou JL, Angel G, Aribot G, Rogier C, Dieje A, Toure-Balde A, Diatta B, Seignot P, Roussilhon C, 1997. Prognostic value of anti-Plasmodium falciparum-specific immunoglobulin G3, cytokines, and their soluble receptors in West African patients with severe malaria. *Infect Immun* 65(8), pp. 3271-6.

Schlichterle IM, Treutiger CJ, Fernandes V, Carlson J, Wahlgren M, 1996. Molecular aspects of severe malaria. *Parasitol Today* 12(9), pp. 329-32.

Sri-Hidajati BS, 2003a. Cerebral malaria I. The role of adhesion molecules in its pathogenesis. *Folia Medica Indonesiana* 39(2), pp. 94-101.

Sri-Hidajati BS, 2003b. Cerebral malaria II. The role of Tumor Necrosis Factor in its pathogenesis. *Folia Medica Indonesiana* 39(3), pp. 157-65.

Taylor-Robinson AW, 1998. Nitric oxide can be released as well as scavenged by haemoglobin, pp. relevance to its antimalarial activity. *Paras Immunol* 20, pp. 49-50.

Weiss G, Wachter H, Fuchs D, 1995. Linkage of cell-mediated immunitty to iron metabolism. *Immunol Today* 16(10), pp. 495-500.