IJCBR

The Involvement of Chemokines and Receptors in Progression of Cerebral Malaria: The Story So Far (Chemokine and Their Receptors' Role in Experimental Cerebral Malaria)

Kalyan Srivastava[1,*], Kaman Srivastava[2]
[1]Scientist, University of Buffalo Research Foundation, University of Buffalo
[2]Assistant Professor, Dr. B.R. Ambedkar Center for Biomedical Research, University of Delhi, New Delhi

***Corresponding Author:**
E-mail: kalsriv@gmail.com

Abstract: *Malaria has long been considered as disease associated with innate and adaptive immune response. The innate immune response, in particular, has widely been studied and implicated to the lethality in malaria. A sequence of immune events contributes to the cerebral manifestation commonly known as cerebral malaria (CM). CM is morbid and mostly irreversible condition which is accompanied by a haywire immune response that destroys the cerebro-protective mechanism of the host. Variety of immune components such as lymphocytes, mono/mac cells, cytokines, chemokines and their receptors have been attributed to this phenomenon. In this review we have attempted to highlight the role of chemokines and their receptors. Using evidences from ECM, we advocate that chemokine receptor such as CCR1, CCR2, CCR5 and CXCR3 contribute towards the pathogenesis. Also the set of chemokines binding to these receptors were over-expressed in CM. Overexpression results in the T Cell and monocyte activation and consequently migration across the blood brain barrier to cause the damage.*

Keywords: *Cerebral Malaria, Chemokines, Chemokine Receptors, CXCR3, CXCL4*

Background

Malaria is a major health nuisance in the endemic zone. Over 250 million people worldwide are infected with malaria each year. Combined with HIV and tuberculosis, malaria is top three major cause of mortality (1). Despite several vaccine trials no protective measure has been medically constituted so far. Vector exclusion and post-infection management are the only answer for this menace. There is ever increasing need for individually addressing each type of outcome of malaria, such as severe anemic malaria (SMA), placental malaria (PM), malaria acute lung injury (MALI) or cerebral malaria (CM). Among these however, the CM is largely independent of therapy status and mostly kills the children less than five years (75000 infants/year). The CM results into massive neurological damage to the patients, with symptoms such as unconsciousness, ataxia, hemiplegia, coma, convulsions and death. The survivors, even a decade later, continue to have episodes of neurological conditions such as impaired learning, seizures and loss of hearing and vision. Therefore, second to the SMA, the condition of CM has devastating health and disability outcomes to the residents of endemic zone. CM also severely affects people traveling to these region such as tourists, business, sports and army personnel as they lack prior immunity against the disease. Although diagnosis of CM is done efficiently, there exists no treatment that specifically applies to CM.

CM is primarily caused by the P. falciparum parasite. In recent years however there has been increase in number of reports of the CM being caused by P. vivax. This, therefore, makes it much important to investigate CM with more attention. Innate immune response in case of malaria infection serves as double edge sword serving as protective as well as

damaging role to the host. The human post-mortem studies have exclusively revealed the presence of leukocytes, monocytes, macrophages and platelets in CM patients' brain. Identical data have been obtained from the experimental cerebral malaria model (ECM) using C57Bl6 mouse infected with P. berghei ANKA. The common feature of CM progression includes the brain blood barrier breakdown causing release of inflammatory cells and chemokines inside the brain (2).

Mouse Mode

Mouse model for cerebral malaria is referred as experimental cerebral malaria (ECM) and has in recent years gained acceptance as a true model for cerebral malaria. This acceptance is due to the high similarity between the histology of tissue from mouse and the human post-mortem sample. This corroboration has made these rather simple model a valuable method for understanding the pathogenesis of disease. The availability of knock-out mice has accelerated the study related to the chemokines role in ECM. Our lab has widely exploited the P. berghei ANKA model and to elucidate the role of CXCL4, monocytes KLF4, protective role of interferon beta in CM and antigen presentation by platelets (3). This model involves intra-peritoneal injection of 106 parasites into the C57Bl6 mice, which develop cerebral manifestation by 5th day and mortality by 6th day. Any death after 10th day is registered as occurring due to the alternative causes (4). Other groups have used P. yoelii 17XL mice injection to the C57bl6 mice as CM model. A mouse model can never completely delineate the human pathogenesis and can either represent lesser or exaggerated response. It is generally believed that immune response in ECM is more intense than its human equivalent. Even between the ECM studies using different parasite, the immuno-pathology might differ. The choice of mice viz. C57bl6 and Balbc may also have difference in immune response. C57Bl6 mice have also been widely used for ECM because most knockout mice have been developed using it (5). These studies highlighted the dependence of CM pathogenesis of these chemokines and provide valuable information about their role (6).

Chemokines, receptors and their role:

It is evident that CM progression is accompanied by a brisk immunological response in the host leading to deleterious outcomes. Both cellular and humoral immunological responses can eventually cause damage to the brain of the host. In ECM model several leukocyte populations including macrophages, neutrophils, lymphocytes, NK Cells and platelets have been found in brain blood micro-capillaries of mice (7). Chemokines are immunologically active polypeptides with molecular weight range of 8-15kDa. So far up to 50 chemokines have been identified in human and mouse. They are classified into four groups based on the positions of key cystiene residues, i.e. C, CC, CXC, and CX3C. The signaling activity by chemokines take place via its binding to the seven-transmembrane spanning G protein coupled receptors (GPCRs) (8). There are nineteen chemokine receptors explained so far. Functionally, chemokines can be characterized as (i) constitutively expressed with immune homeostatic role (ii) inducibly expressed with immune protective role (iii) a hybrid of constitutive and inducibly whose level are increased upon immune signal (9).

Our and other groups have illustrated the role of CD8(+) T Cells in deleterious events of ECM (6; 10); 11; 12). The studies demonstrate that CD8(+) T cells of diverse specificity induced during ECM infection share many characteristics such as expression cytolytic markers (gamma interferon [IFN-γ], granzyme B) damage the blood-brain barrier in vivo. These events therefore highly correlate CD8(+) T Cells proliferation, activation and sequestration to the blood brain barrier breakdown. The activation of T Cells takes place via chemokine receptors such as CCR5 and CXCR3. The chemokines and receptors together act as chemo-attractant for T Cells and are involved in migration and recruitment of lymphocytes and monocytes into the brain. The cytokines such as IL-1, IL-6, IL-8, IL-10 and IL-12 have also been implicated in the pathogenesis of human CM and SM and act in synergy with the

chemokine to carry out the cerebrovascular damage (13).

Data from microarray studies on ECM samples have also revealed the important role played by the chemokines and their receptors in pathogenesis of the disease (14). However, among different classes of GPCR receptors CCR1, CCR2, CCR5 and CXCR3, have been found to play most critical role in the CM (15). Among these CCR1, highly implicated in cancer, is a CC type of chemokine receptor. The ligands of this receptor include CCL3 (MIP-1-alpha), CCL5 (RANTES), CCL7 (MCP-3), CCL9 (MIP-1-gamma), CCL14 (HCC-1), CCL15 (MIP-1-delta), CCL16 (HCC-4) and CCL23 (MIP-3) (16). Furthermore, the ligands binding these receptors such as CCL3, CXCL5 and CCL9 have highly elevated expression in ECM using any type of mouse model (17). The knock out mice from these three chemokine survive upon ECM study. No study has been performed for CCR1 knockout mouse although it is available with most of the vendors. A further investigation needs to be performed to understand the other CCR1 binding chemokines such as CCL7, -14, -15, -23. Another chemokine receptor that has been associated with CM and studied using ECM is CCR5. This receptor shows enhanced survival (18). This chemokine receptor has been highly reviewed for its association with HIV entry into the target cells. Common ligands that bind to the CCR5 includes CCL3, CCL4, CCL5, CCL3L1, CCLl4. As indicated above among these CCL3 and CCL5 have already been studied in ECM. The other chemokine viz CCL3L1 and CCLl4, therefore are awaiting studies using knockout mice. Receptor CCR5 is also expressed in the brain, indicating their important role in cerebral inflammation in response to different diseases. CCR5 also interacts with the other GPCRs to carry out its function and blocking these interaction can abolish its function.

The chemokines CXCL4, CCL9, CCL10 and CCL11 can share a common chemokine receptor known as CXCR3 (19). Our study has previously demonstrated that CXCR3 receptor is critically important for ECM progression. This and subsequent studies have clearly indicated that CXCR3-/- mice were 100% protected against ECM. This protection was independent of parasite load. Suggesting that onset of diseases alone triggers this pathways. An important chemokines released exclusively by the platelets, PF4 or CXCL4, bind specifically to the CXCR3 receptors on monocytes and other target cells. CXCR3 are highly expressed in T Cell, therefore, indicating that PF4 binding to immune cells incite their migration across brain cerebral microvessel(3;20). CXCL4 are released by the platelets in variety of physiological and pathological conditions including malaria. Later investigation also revealed the direct involvement of CXCL9 and CXCL10 in ECM which can be ameliorated by administration of IFNbeta (21). Another important receptor studied using knockout mice and ECM is CCR2 (22). CCR2 depleted mice do not have improved survival and are increasingly susceptible to CM. CCR2 coding gene expresses two isoforms of a receptor for monocyte chemoattractant protein-1 (CCL2), a chemokine which specifically mediates monocyte chemotaxis and is involved cognitive processes (23). Therefore, in human it can be assumed that onset of CM might interfere with CCR2 expression or activity. Therefore, it is imperative to discuss these subfamily of chemokine receptors and ligands binding them in CM and other malaria pathogenesis. Table 1 provides a summary to describe of these receptors and their roe in CM. numerous other chemokines receptors such as CXCR1, CXCR2 and their ligands await recognition for their role in CM. Most of data obtained are from mouse model and need to be individually validated. Malaria related factors such as iRBC sequestration to the cells of different tissues origin and immune cells, exo-erythrocytic parasites and hemozoin formation in malaria can contribute to the increase in chemo-attractant cytokines and chemokine formation. Score of data illustrate that CXCL9, CXCL10 and CXCL11 are produced in diverse pathological conditions effecting central nervous system. Enhanced attention has been given to the function of these chemokines and their common receptor CXCR3 in the CNS inflammation in other diseases. These chemokines act via CXCR3 and are highly associated with CD8+ and CD4+ T Cell activation (24). It is therefore acceptable that T Cell activation

and extravasation to the brain in CM patients can occur in an identical fashion.

Conclusion

As evident from the table 1 several chemokines receptors and the corresponding, binding chemokines critically require close investigation using ECM studies. The studies at this stage must include the conditional knock out with immune cells lacking these receptors. The receptor CCR1 needs a closer investigation using global knockout mouse model. This would help in further understanding their role in disease progression and possible therapeutics development. Further studies must be performed using clinical sample to systematically study the role of CXCR3, CCR5 and CCR1 receptor binding. Another set of receptors that need investigation include CXCR1 and CXCR2, expressed mostly on neutrophils, as they have a large set of chemokine binding to them. The cerebral manifestation has mostly been evaluated by studies such as extravasation of Evan's Blue dye, histology, visual evaluation and electrological studies. These methods although highly accepted present their own pitfalls. Advancement in imaging techniques and murine adaptation such as laser doppler, contrast MRI and PET method can help in closely evaluating the progression of ECM. So far no effort has been made to develop a common therapy for the CM. Most of the treatments relies totally over the amelioration of fever and lowering of parasitemia and waiting for cerebral damage to recover. In this regard the chemokine and their receptors can serve as a great starting point for a clinical trial in endemic zone. It can therefore be proposed to perform a trial engaging orphan drugs developed for HIV targeting CCR5 to be tested for CM. Our study has highlighted the effect of IFN beta, a multiple sclerosis related neuro-protective agent, in CM. IFNbeta suppresses the expression of many deleterious chemokines in CM as well as neuro-degenerative diseases. With cost of IFNbeta reduced, it can also be an important drug against CM in endemic zone.

Declaration

The work was not funded by any current agency. Animal and human studies were performed according to the institutional guidelines.