Review Article

Protective Function of Intermediate T Cells against Malaria Infection in Mice with Different Genetic Background

Hanaa Y. Bakir* and Amal M. Elmatary

Department of Parasitology, Faculty of Medicine, Assiut University, Egypt

*Correspondence Info:
Dr. Hanaa Bakir,
Department of Parasitology,
Faculty of Medicine, Assiut University, Egypt
E-mail: hanaabakeer@yahoo.com

Abstract
This review proposed the possibility that malaria protection might be achieved through intermediate T cells, which is one of the constituents of innate immunity. Intermediate T cells here in have been assessed to represent the flexibility of the immune system with the alteration of genetic background in different mice groups during malaria infection. Intermediate T cells are unique subset of unconventional lymphocytes that express an intermediate (int) density of T Cell Receptor-CD3 (TCR-CD3) complex on its surface (i.e. TCR<sup>int</sup> cells), which distinguishes them from conventional T cells (thymus-derived T cells or TCR<sup>abh</sup> cells). TCR<sup>int</sup> cells comprise from two subsets with a distinct phenotype Natural killer (NK) 1.1<sup>+</sup> subset (NKT) cells and NK1.1<sup>−</sup> subset. The protective function of TCR<sup>int</sup> cells was challenged by infecting mice from different genetic background with malaria. There was a prominent expansion of intermediate T cells in the liver of the mice even the genetically deficient one, which is characteristic of an innate immune response. In parallel with such expansion, no expansion is seen in conventional T cells due to severe thymic atrophy. Thus, intermediate T cells are emerging as an important subset of lymphocytes; with a protective role that is modulated according to the genetic background of the mice. Added to that establishment of an effective immune defense network to modulate the reciprocal regulation between conventional and unconventional T cells.

Keywords: Malaria, Mice, and Intermediate T Cells

1. Introduction
Malaria remains an important cause of morbidity and mortality. Approximately 207 million malaria cases and 627 000 malaria-related deaths were reported globally in 2012. The greatest toll is expected in sub-Saharan Africa, where over 80% of all malaria episodes and 90% of all malaria-related deaths occur. The huge malaria burden in sub-Saharan Africa has been partly attributed to the presence of efficient vectors that maintain high levels of transmission<sup>1</sup>-<sup>3</sup>. Malaria life cycle involves two hosts, the insect vector and the intermediate mammalian host. During its complex, multi-stage life cycle, the malaria parasite not only expresses a great variety of proteins at different stages, but these proteins also keep changing. As a result, a natural infection with malaria parasites leads only to a partial and short-lived immunity that is unable to protect the individual against a new infection.<sup>4</sup>

Although the advent of DDT and chloroquine led to the belief that eradication was possible, the spread of parasites and insects resistant to the drugs and insecticides has led to a resurgence of the parasite in economically disadvantaged countries.<sup>5</sup> This worsening situation has called for the development of new control measures, of which vaccines have been a priority since the late 1970s. However, the complex interplay of parasite proteins with the immune system of the host has also made it difficult or even impossible to develop an effective vaccine against the disease. Up to now, no vaccine formulation with sufficient efficacy against malaria parasite has been developed.<sup>5</sup> Reasons for these failures are mostly due to the complexity of the malaria parasite.

2. Natural or innate immunity against malaria
Innate immunity against malaria is an inherent refractoriness of the host that prevents the establishment of the infection or an immediate inhibitory response against the introduction of the parasite. The innate immunity is naturally present in the host and is not dependent on any previous infection.<sup>6</sup>-<sup>7</sup> Acute malarial infection induces immediate, non-specific immune response that tends to limit the progression of disease. The humoral and cellular mechanisms of this ‘nonspecific’ defense are poorly defined. Extrathymic T Cells and autoantibody producing B cells or TCR<sup>+</sup> cells have been considered as the prime movers of this response. Natural killer (NK) cells are found in blood, in secondary lymphoid organs as well as in peripheral non-lymphoid tissues. Related cell types, probably playing a role in innate malaria immunity, are the Natural killer T (NKT) cells in the mice carry both the NK1.1<sup>+</sup> surface marker and T cell receptors (TCR).<sup>7</sup> NK cells have been shown to increase in numbers and to be able to lyse P. falciparum-infected erythrocytes in vitro. NK cells in peripheral blood produce interferon-gamma in response to Plasmodium infected erythrocytes, leading to parasiticidal macrophage activation. This may be of greater importance for innate malaria immunity than their potential to lyse infected host erythrocytes.<sup>8</sup>-<sup>9</sup>

Cells of innate immunity are also important in the initiation and development of adaptive immune responses. NK cells induce the production of the pro-inflammatory chemokine Interleukin-8, which in turn plays its role in the recruitment and the activation of other cells during malaria infection. Dendritic cells, macrophages, gamma delta T cells and NKT cells also sense the presence of the parasite and participate in the immune response.<sup>9</sup>-<sup>10</sup> NKT cells are potent inhibitors of liver stage parasite replication in mouse malaria systems in vitro. Malaria infection gives rise to strongly elevated blood concentrations of non-malaria-specific immunoglobulin, but the importance of the underlying polyolonal B-cell activation for innate immunity is not known.<sup>10</sup>-<sup>12</sup>

3. Generation and differentiation of intermediate T Cell Receptor (TCR<sup>int</sup>) cells
It is well established that the thymus is an essential organ for the support of T-cell differentiation. However, some T cells termed extrathymic T cells have been found to differentiate without such support by the thymus. The major sites of these T cells are the liver and intestine. The liver has been found to be one of the important hematopoietic organs even after birth.<sup>13</sup> Namely, adult liver still comprises e-ki<sup>−</sup> stem cells and gives rise to extrathymic T cells, NK cells, and even granulocytes. Extrathymic T cells generated in the liver of mice are TCR<sup>int</sup> cells. Abo et al.<sup>14</sup> have revealed that the subsets of TCR<sup>int</sup> cells comprise cells with a distinct phenotype. The interleukin 2 Receptor(II-2)p<sup>−</sup>NK1.1<sup>−</sup> subset consists mainly of double-negative (DN)/CD4<sup>−</sup>8 cells and
CD4 cells, whereas the IL-2Rβ NK1.1 subset consists mainly of CD8− cells. All T cells in congenitally athymic nude mice (mice without thymus) are TCRαβ cells; it is obvious that TCRγδ cells are generated extrathymically.

In parallel with the extrathymic pathway in the liver, TCRαβ cells are also generated through an alternative intrathymic pathway. Although a few TCRαβ cells are dispersedly present in the cortex region, the majority of these T cells exist in the medullary region. In contrast, the mainstream of T-cell differentiation for the generation of conventional T cells occurs in the cortex region. Similar to the case of extrathymic T cells in the liver, TCRγδ cells in thymus are modulated by malarial infection in mice with different genetic background. To precisely determine the genetic background, the number of intermediate T cells in the liver of mice with malarial infection, we found that the production of autoantibodies such as γ2 were infected with a non-lethal strain of Plasmodium (P. yoelii 17XL). Malaria infection is accompanied by severe thymic atrophy and the proportion of TCRαβ+CD3− cells rather decreases in the periphery. Indeed, there is no protective effect of TCRαβ+CD3− cells when these T cells isolated from mice recovered from malaria are injected into irradiated mice.

Although intermediate T cells are few in number in youth, they gradually increase in number with aging. Even in youth, the number and function of intermediate T cells are elevated under conditions of stress, infection, malignancy, pregnancy, autoimmune disease, chronic graft-versus-host diseases, etc. Under these conditions, the mainstream of T-cell differentiation in the thymus, which produces conventional T cells, is rather suppressed. Therefore, reciprocal regulation between extrathymic T cells and thymus-derived T cells might be present.

3.1 Characteristics of intermediate T cells

Intermediate T cells are intimately associated with innate immunity. Although intermediate T cells have slightly distinct properties depending on the sites, they consistently express IL-2Rβ chain on the surface. Similar to the case of NK cells, intermediate T cells have the IL-2Rαββ phenotype; i.e., an intermediate affinity IL-2R. In contrast, conventional T cells have the IL-2Rαββ phenotype under resting conditions but have the IL-2Rαββ phenotype, i.e., a high affinity IL-2R under activated conditions. In other words, NK cells and extrathymic T cells consistently express intermediate affinity IL-2R under usual conditions. Reflecting this situation, NK cells and extrathymic T cells respond quickly to corresponding antigens. It is speculated that conventional T cells acquired a resting state in phylogenetic development but NK cells and extrathymic T cells did not. This situation indicated that more primitive lymphocytes respond more quickly (NK cells > TCRαβ cells > TCRγδ cells). Among these, however, the magnitude of response is the greatest in TCRγδ cells.

Both NK cells and intermediate T cells are granular lymphocytes in morphology. In case of intermediate T cells in the liver, they have an intermediate density of TCR-CD3 complex on the surface. On the other hand, conventional T cells are estimated to be TCRγδ cells. Watanabe et al. identified TCRαβ cells in mice by applying two-color staining for CD3 and IL-2Rβ, they demonstrated that NK cells were identified as IL-2RαβCD3+, TCRαβ cells as IL-2Rβ+CD3+, and conventional T cells as IL-2Rβ−CD3+. In other words, both NK cells and TCRαβ cells consistently express IL-2Rβ, while conventional T cells lack the expression of IL-2Rβ.

3.2 Role of intermediate T cells in protecting the body from plasmodium

The body seems to be protected against malaria by innate immunity. This concept supported by the fact that malarial infection induces a prominent expansion of intermediate T cells (IL-2Rβ+TCRαβ−cells) in the liver. No expansion is seen in conventional T cells (IL-2Rβ+TCRαβ−cells). When TCRαβ cells isolated from liver are divided into three subsets, i.e., conventional T cells, intermediate T cells, and NK cells, it is clear that NK cells are few in number in youth, they gradually increase in number with aging. Even in youth, the number and function of intermediate T cells are elevated under conditions of stress, infection, malignancy, pregnancy, autoimmune disease, chronic graft-versus-host diseases, etc. Under these conditions, the mainstream of T-cell differentiation in the thymus, which produces conventional T cells, is rather suppressed. Therefore, reciprocal regulation between extrathymic T cells and thymus-derived T cells might be present.
was retarded in B6 mice, the number of lymphocytes was comparable to that found in the other strains of mice. The absolute numbers of IL-2RβCD3int cells were calculated (Fig. 2b), there were also marked increases in the numbers of IL-2RβCD3int cells in DBA/2 and BDF1 mice during recovery phase (P < 0.05).

Figure 2. A: Number of lymphocytes yielded by the liver. B6, BDF1 and DBA/2 mice were used for malarial infection. The acute phase was day 7 in all strains, whereas the recovery phase was day 28 in B6 mice and day 20 in DBA/2 and BDF1 mice. *P < 0.05

B: Identification of the absolute number of IL-2RβCD3int cells. *P < 0.05

To identify lymphocyte subsets, two-color staining for CD3 and IL-2Rβ was carried out; TCRβ+IL-2Rβ+ cells expanded more prominently in DBA/2 and BDF1 mice (H-2d carrying strain) than in control B6 mice (H-2b) (indicated by arrows Fig 3).

Figure 3: Identification of IL-2Rβ+CD3int cells. Two-color staining for CD3 and IL-2Rβ were conducted in the liver. Arrows indicate the expansion of IL-2Rβ+CD3int cells. Numbers in the figure represent the percentages of fluorescence-positive cells in the corresponding areas. Representative results of three experiments are depicted.

The production of various cytokine mRNA during malarial infection was compared between B6 mice and BDF1 mice by Reverse transcription polymerase chain reaction (RT-PCR) (Fig. 4). In addition to IFN-γ and IL-4 mRNAs, the levels of inflammatory cytokine (i.e. IL-12p40, IL-6, TNF-α, IL-1β and IL-10) mRNAs increased in both B6 mice and BDF1 mice. The most marked difference was in the decrease of TNF-α and IL-7 mRNA/s during the recovery phase in BDF1 mice. In contrast, G-CSF mRNA increased in BDF1 mice. The level of GM-CSF mRNA was low in both strains of mice.

Figure 4. Signs of cytokine mRNAs in the liver tissue of B6 and BDF1 mice during malarial infection. Integrity of RNA was confirmed by the sign of G3PDH. Representative results of three experiments are depicted.

These results suggest that DBA/2 mice, which have poor functions of conventional T cells, show rather strong functions of unconventional T cells. This genetic defect seems to induce a compensatory function of primitive lymphocytes that is important for the acquisition of resistance against malarial infection.
4.2 Athymic nude mice

Mannoor et al using congenitally athymic nude mice B6-nu/nu mice carry only TCRαβ-IL-2Rβ+ cells but lack TCRγδ-IL-2Rβ+ cells. The major expanding T cells were NK1.1 IL-2Rβ⁺CD3⁺ cells when athymic nude mice were infected with malaria and subsequently recovered from it. Moreover, by cell transfer experiments, these NK1.1 IL-2Rβ⁺CD3⁺ cells were found to have the ability to protect the mice from malaria if such lymphocytes were isolated from the liver of mice that had recovered from malaria. These results suggest that the protection from malarial infection might be consequent of immunological events achieved by intermediate T cells.

4.3β2-microglobulin-deficient (β2m (−/−) mice

Another study, conducted by Taniguchi et al using β2-microglobulin-deficient (β2m (−/−)) mice, which lack CDS⁺ cytotoxic T cells and NK cells. Deficiency of NKT cells occur due to the absence of both MHC class I antigens and CD1d antigens (MHC class I-like molecule with β2-microglobulin component). The results showed that β2m (−/−) mice showed a level of parasitaemia similar to that of B6 mice and were able to recover from malaria infection. In other words, mice were found to recover from malaria in the absence of CDS⁺ T cells and NK cells. A major expansion of IL-2Rα⁺ TCRβ⁺ cells was common in B6 and β2m (−/−) mice. These results suggest that there is a compensatory phenomenon in the immunity of β2m (−/−) mice.

4.4 AIM (Apoptosis Inhibitor expressed by Macrophages) deficient mice

In a recent study, Li et al. used AIM (Apoptosis Inhibitor expressed by Macrophages) deficient (AIM−/−) mice. The AIM is exclusively secreted by tissue macrophages and is not involved in apoptosis in mice. The AIM rapidly increases in response to inflammatory stimuli, inhibits apoptosis of thymocytes and induces resistance to apoptosis in various immune cells, such as antigen-presenting cells (macrophages [including Kupffer cells]) and NK cells. The AIM−/− mice showed a level of parasitaemia similar to that of B6 mice and were able to recover from malaria infection. These results suggest that the protection from malarial infection might be consequent of immunological events achieved by intermediate T cells.

5. Conclusions

The obvious conclusion from the data discussed here is that the intermediate T-cells are emerging as an important subset of unconventional lymphocytes, which modulate themselves to play an active protective role in host defense. In this respect, these results indicate that primitive T cells, namely intermediate T cells may be much more activated in some groups of mice than the others and therefore resistance to malaria infection occurs in mice which may have defective function in some immune cells. Because of this situation, the immune system has to be switched from the usual system to the emergency system associated with NK cells and unconventional T cells. Based on the data discussed above, it is clear that a better knowledge of the mechanisms governing innate immunity based on genetic background will provide new clues for understanding how the immune system works in malaria infection and helps in designing therapeutic strategies.

References


