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Experimental cerebral malaria: Possible new mechanisms in the TNF-induced microvascular pathology

Summary

In order to contribute to the prevention of malaria morbidity and mortality, especially in endemic zones, we have carried out a series of studies on cytokine interactions in an experimental model of cerebral malaria (CM). This rapidly lethal syndrome develops in some strains of mice, upon infection with Plasmodium berghei ANKA (PbA). A crucial mediator of neurovascular lesions appears to be TNF, found in high amounts in relation with cerebral complications, in both experimental and human CM. In experimental CM, in vivo injections of anti-cytokine antibodies have been used to analyze the cascade of reactions leading to brain vascular damage. In this review, we will focus on 1.) the interplay of cytokines responsible for TNF overproduction in experimental malaria, therefore delineating the subset of T cells whose activation can lead to pathology, and effector mechanisms of neurovascular lesions characteristic of mouse cerebral malaria, with recent findings that appear to involve an unexpected cell type, the blood platelet.

Malaria remains a major problem of public health at the world level. Morbidity and mortality of malaria result from complications that include cerebral malaria (CM) and severe anemia. In addition to clinical and epidemiological studies allowing for the assessment of markers of disease activity, an experimental approach towards malarial pathology is required. One of the interests of the experimental approach is the possibility to gain knowledge in the fine mechanisms of disease, and to define new markers of disease severity.

In order to understand better the mechanisms of CM, we have used both in vivo and in vitro models. The in vivo model is suitable for direct intervention studies, while the in vitro studies, dealing with the target cell of neurovascular lesions, namely the endothelial cell, allows for a functional dissection of the mechanisms of tissue lesion. We have studied cytokine interactions in an experimental model of vascular pathology: cerebral malaria (CM). This rapidly lethal syndrome develops, in some strains of mice, upon infection with Plasmodium berghei ANKA (PbA). A crucial mediator of neurovascular lesions appears to be TNF, found in high amounts in relation with cerebral complications, in both experimental and human CM. In experimental CM, in vivo injections of anti-cytokine antibodies have been used to analyze the cascade of reactions leading to brain vascular damage.

In this review, we will focus on 1.) the interplay of cytokines responsible for TNF overproduction in experimental malaria, therefore delineating the subset of T cells whose activation can lead to pathology, and 2.) effector mechanisms of neurovascular lesions characteristic of mouse cerebral malaria, with recent findings that appear to involve an unexpected cell type, the blood platelet.

Cytokine interplay in cerebral malaria: Evidence for preferential expansion of T cells of the Th1 subset in genetically susceptible mice

The immunological responses that contribute to resistance versus susceptibility to bacterial and parasitic infections seem to depend upon the presence of functionally distinct CD4+ T cells: T helper (Th)1 and Th2 cells. Th1 cells, which release
interferon-gamma (IFN-\(\gamma\)) and IL-2, appear to participate in protective responses, whereas Th2 cells, which release IL-4, IL-5, IL-6 and IL-10, are more often associated with pathology (for review see 3). In the murine model of leishmaniasis, for example, it has been demonstrated that strains susceptible to lesions show a predominant Th2 response while resistant strains of mice display a Th1 response 4. Th1 cells also participate in resistance to Trichinella infection via release of IFN-\(\gamma\) while Th2 cells contribute to susceptibility by production of IL-4 5. In malaria, using the mouse model of Plasmodium chabaudi infection, protective immune responses involving IFN-\(\gamma\) were shown to be a result of a predominant Th1 response 6. Conversely, in the mouse model for CM induced by infection with Plasmodium berghei ANKA (PbA), IFN-\(\gamma\) seems to play a role in susceptibility inasmuch as anti-IFN-\(\gamma\) monoclonal antibody treatment in vivo leads to protection against CM 7. In previous experiments we showed that CM is strictly dependent upon the presence of CD4+ T cells 8; indeed, depletion of CD4+ T cells completely protects infected CM-susceptible (CM-S) mice against the cerebral complications associated to CM. A crucial mediator of these neurovascular lesions appears to be tumor necrosis factor/cachectin (TNF) 9, released by macrophages stimulated by CD4+ T cells via a cascade of cytokines that includes IFN-\(\gamma\), IL-3 and GM-CSF 10.

In order to examine the relationship between susceptibility and resistance to CM and Th1 versus Th2 responses, we examined cytokine mRNA expression in vivo in brain and spleen from mice that are susceptible (CM-S) or resistant (CM-R) to cerebral malaria. CBA/J (CM-S) and BALB/c (CM-R) mice were compared before and 7 d after infection with PbA. Expression of cytokine mRNAs was correlated to in vitro cytokine production profiles in lymph node and spleen cells from uninfected and infected mice in response to parasitized red blood cells and crude malarial antigens. Our results provide evidence that susceptibility to cerebral malaria is accompanied by the up-regulation of IFN-\(\gamma\) gene expression and in vitro production, in response to specific malarial antigens. The expression of TNF-\(\alpha\), IL-1, and IL-6 mRNAs was also found to be increased in the spleen of infected mice as compared to that of uninfected mice. There was no difference between CM-S and CM-R mice, except for TNF-\(\alpha\) mRNA, which accumulated in higher amounts in the brain of infected animals with CM than in those with similar level of infection but without CM. Conversely, we found that the expression of two cytokines that are potentially able to antagonize TNF-\(\alpha\) effects, IL-4 and TGF-\(\beta\), was significantly downregulated at the time of CM. IL-4 gene expression in vivo appeared to be turned off, as no transcript was detected even when assayed by PCR. Upon restimulation by crude malarial antigens in vitro, production of IL-4 was significantly decreased in infected CM-S mice. The levels of IL-3 produced in response to malarial antigens were found to be comparable between both infected CM-R and CM-S mice and no detectable IL-5 was found in spleen cell cultures, either unstimulated or stimulated with parasitized red blood cells or malarial antigens 11.

It had previously been shown that treatment in vivo with anti-IFN-\(\gamma\) mAb was able to protect PbA-infected mice from CM and to prevent the associated TNF overproduction 7. Thus, beyond the question of the requirement of IFN-\(\gamma\) in CM, there remained the question of whether susceptibility to CM correlated with a particular pattern of cytokine production. Genetic susceptibility to CM was found to be linked to a higher IFN-\(\gamma\) production capacity. Moreover, at the onset of cerebral complications, IFN-\(\gamma\) mRNA significantly accumulated in the brain. These data correlate with the results obtained by Waki et al. 12 who showed, in a mouse model of malaria induced by a virulent strain of P berghei NK 65, that CD8+ T cells were capable of producing IFN-\(\gamma\) and thus induce the production of TNF-\(\alpha\) in the liver. In this particular model, an anti-CD8+ T cell treatment led to prolonged survival while anti-CD4+ T cell treatment had no effect. In our model, the CD8+ T cell subset does not appear to exert a protective role since depletion of CD8+ T cells in CM-R mice did not lead to the development of CM (unpublished data). These results should be discussed in relation with previous observations of a higher capacity of malaria-specific IFN-\(\gamma\) production capacity in non-immune individuals compared to that of immune subjects 13. Since it is known that non-immune individuals, such as children in endemic areas or adult visitors from non-endemic areas, are particularly prone to CM, these data suggested that a naive immune system is associated with a higher susceptibility to develop CM. If IFN-\(\gamma\) over-production is indeed associated to susceptibility, this would hint at the involvement of a Th1-like response. A Th1-like pattern of cytokine synthesis associated to susceptibility has also been suspected in other infectious diseases such as Theiler’s murine encephalomyelitis 14. This is in sharp contrast to the situation observed in other parasitic diseases such as schistosomiasis and leishmaniasis in which Th2 rather than Th1 responses correlate with pathology. In the murine model of schistosomiasis, experiments had suggested that T cells from vaccinated mice, after stimulation with specific antigen or mitogen, responded primarily with Th1 cytokines, whereas lymphocytes from chronically

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