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HOST IMMUNE RESPONSE AND IMMUNOPATHOLOGY IN MALARIA

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Recent progress in molecular biology has allowed the elucidation of the fine structure of many plasmodial antigens, whose genes and amino acid sequences are becoming available. Better knowledge of the circumsporozoite (CS) proteins of *Plasmodium falciparum, P. vivax, P. knowlesi* and *P. cynomolgi* (Nussenzweig & Nussenzweig, 1985), the high molecular weight antigen of the merozoite surface of *P. falciparum*, and the *P. falciparum* ring infected erythrocyte surface antigen (Anders, 1985) represent some examples of the major achievements in this field. Such antigens, which contain tandemly repeated amino acid sequences, are now considered as possible candidates for malaria vaccines. In fact, peptides consisting of the repetition unit of these antigens have been now produced using both chemical synthesis and recombinant DNA technology. These peptides are now entering an operational vaccination trial phase.

The use of synthetic or engineered malaria molecules as potential vaccines requires a series of studies in order to evaluate the actual effectiveness of such molecules in terms of protectivity and the possible adverse effects in terms of triggering of immunopathological reactions. In fact, many immune phenomena of the malaria infection remain unknown. Moreover, some genetic factors could possibly interfere with an efficient immune response towards parasite antigens employed as vaccines.

Little is still known about the fine mechanisms underlying the development of a state of immunity, leading to recovery, as well as those involved in the immune response to the parasite antigens and consequently to the potential peptide vaccines. Moreover, the precise pathogenesis of the major (cerebral, renal and hematological) complications of malaria infections remains controversial. The immunopathological nature of these complications has already been suggested in humans and in experimental models (Cohen & Lambert, 1982). Because of these advances in the field of malaria vaccines, it may be relevant to question the importance of the individual patterns of host immune responses in the achievement of immunity or in the expression of immunopathological complications of malaria.

These questions have been addressed in our laboratories by studying certain aspects of the immune response to malaria parasite antigens in two experimental models. Firstly, the role of the genetic background in the immune responsiveness to synthetic sporozoite peptides was investigated in mice bearing different major histocompatibility complex (H-2) haplotypes. Secondly, the role of the immune response to malaria antigens was considered in the pathogenesis of cerebral complications of murine malaria.

CAN GENETIC RESTRICTION LIMIT THE EFFECTIVENESS OF MALARIA VACCINES? GENETIC CONTROL OF THE IMMUNE RESPONSE TO SYNTHETIC CIRCUMSPOROZOITE REPETITIVE EPITOPE OF *P. FALCIPARUM* IN MICE

Malaria sporozoites possess a main antigen expressed on their external coat, the so-called circumsporozoite (CS) protein. Anti-sporozoite antibodies react with the CS protein in a species- and stage-specific manner, also inducing the loss of infectivity of sporozoites. Indeed, in experimental animal models and in human volunteers irradiated sporozoites were shown to confer sterile immunity (Cochrane et al., 1980). The CS proteins so far characterized at gene level consist of an immunodominant epitope repeated several times and flanked by unrepeated amino acid sequences (Nussenzweig & Nussenzweig, 1985). The *P. falciparum* CS protein consists of four amino acids (Asn-Ala-Asn-Pro = NANP) repeated 37 times (Dame et al., 1984; Enea et al., 1984) and very well conserved in all the isolates investigated (Weber & Hockmeyer, 1985; Zavala et al., 1985a). Moreover, many (if not all) anti-sporozoite monoclonal and polyclonal antibodies so far produced recognize specifically the $(NANP)_n$ epitope. Taken together, these findings suggest that such an epitope can serve as a malaria vaccine.

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In fact, this repeated sequence was produced both by chemical synthesis (Ballou et al., 1985; Zavala et al., 1985b) and by recombinant DNA technology (Young et al., 1985). These peptides coupled to carrier proteins were shown to be immunogenic in animals, inducing the production of antibodies recognizing specifically the *P. falciparum* sporozoites and able to inhibit their penetration into cultured hepatic cells and their maturation into exoerythrocytic forms (Mazier et al., 1986).

In our laboratories, the immunogenicity of a novel synthetic peptide consisting of 40 (Asn-Ala-Asn-Pro) repeats, $(NANP)_{40}$, was studied in mice immunized with this peptide not conjugated to carrier proteins. It was shown that $(NANP)_{40}$ was able to induce the production of high titers of anti-*P. falciparum* sporozoite antibodies (Del Giudice et al., 1986a) and a peptide-specific T-cell proliferation (Togna et al., 1986) in C57BL/6 ($H\text{-}2^b$) mice. Athymic C57BL/6 nu/nu mice were completely unresponsive (Del Giudice et al., 1986a). However, when 14 strains of mice bearing nine different H-2 haplotypes were immunized with carrier-free $(NANP)_{40}$, it was observed that *only* $H\text{-}2^b$ mice were able to produce these antibodies, non-$H\text{-}2^b$ mice being completely unresponsive. Then, using recombinant mice with the B10 genetic background, the anti-$(NANP)_{40}$ antibody response was shown to map to the $I\text{-}A^b$ region. This finding was confirmed by the lack of antibody production in B6C.$H\text{-}2^{bm12}$ mice which carry a mutation at the level of three amino acids in the chain of the $I\text{-}A^b$ (Del Giudice et al., 1986a). Furthermore, T-cell clones derived from C57BL/6 mice and specific for $(NANP)_n$ peptides were able to grow only in the presence of antigen-presenting cells bearing the $I\text{-}A^b$ haplotype. This growth was inhibited by the addition of an anti-$I\text{-}A^b$ monoclonal antibody to the cultures (Togna et al., 1986). Finally, this highly selective genetic restriction was overcome when $H\text{-}2^b$, $H\text{-}2^d$, and $H\text{-}2^k$ mice were immunized with $(NANP)_{40}$ conjugated to keyhole limpet hemocyanin as a carrier protein (Del Giudice et al., 1986a).

Our results demonstrate that the immune response against the immunodominant repetitive epitope of *P. falciparum* CS protein in mice is under strict genetic control. Only $H\text{-}2^b$ mice produced anti-$(NANP)_{40}$ antibodies and had a T-cell repertoire specific for such an epitope. Moreover, it is well known that relatively high percentage of children under 10 years of age living in malaria endemic areas do not have detectable anti-*P. falciparum* sporozoite antibodies (Tapchaisri et al., 1983; Zavala et al., 1985c; Del Giudice et al., 1986b, c), while at the same time presenting high antibody titers against asexual *P. falciparum* blood stage antigens (Del Giudice et al., 1986c). In addition, it was observed that in children living in the same village in malaria-endemic countries, the frequency of anti-$(NANP)_{40}$ antibodies was very different in spite of the similarities in the spleen rates, parasitemias, anti-*P. falciparum* blood stage antibodies and in their exposure to mosquitoes (Del Giudice et al., 1986c). These observations may reflect a possible role of genetic background in natural immunization against the *P. falciparum* CS immunodominant epitope. Because of the fact that this genetic restriction can be overcome by using carrier proteins, it is conceivable that a MHC restriction could limit the capacity of some vaccinated individuals to develop an adequate T-cell response specific for the repetitive epitope. In these individuals the presently proposed conjugated vaccines may be efficient in inducing an antibody response but not T cells specific for the repetitive epitope.

CAN MALARIA VACCINES ENHANCE IMMUNOPATHOLOGY? ANALYSIS OF THE CELL SUBSET AND OF THE SPECIFICITY OF ANTIBODIES ASSOCIATED WITH THE DEVELOPMENT OF MURINE CEREBRAL MALARIA

The pathogenesis of neurological complications of *Plasmodium falciparum* infection remains poorly understood although various mechanisms have been proposed. First, endothelial lesions secondary to proliferation of parasites in deep tissues (Knisely, 1961; Wash, 1979), sequestration of parasitized red blood cells in capillaries (Yoeli & Hargreaves, 1974), a hyperergic reaction of the central nervous system to antigenic challenge (Toro & Roman, 1978), effects of a T cell-mediated cellular immune reaction (Wright et al., 1981; Finley et al., 1982) or a T cell-dependent humoral reaction. The latter would be responsible for the production of circulating immune complexes (CIC) (Contreras et al., 1980) that stimulate the lysosomal exocytosis of monocytes (Rest & Wright, 1979) or of IgG-IgM cryoglobulin as a mediator (Adam et al., 1981) and, on the other hand, for the triggering of antibody- or CIC-dependent effector mechanisms. The involvement of disseminated intravascular coagulation (D.I.C.) in cerebral malaria has also been suggested (reviewed in Wilson et al., 1982).

In this study, we focussed our attention on the role of immune response at the cellular and humoral levels in the development of cerebral malaria. The mouse model of cerebral malaria after infection with *Plasmodium berghei* asexual blood stages was analyzed. In this experimental

model. a cumulative mortality of about 90% between day 7 and day 15 of infection was observed in CBA mice. which appeared to be genetically susceptible to the development of neurological lesions. A particularly important feature of this model is that cerebral signs occurred when anemia was moderate and the parasitemia relatively low (Grau et al., 1986). In addition, several clinical and histopathological parameters were found to be similar to those observed in patients with cerebral malaria (Jerusalem et al., 1983; Grau et al., 1986).

Immune mechanisms, and particularly T-cell mediated immunity have been implicated in the development of cerebral complications by the studies of Wright et al., 1981 and Finley et al., 1982. In murine models, the concept that helper T ($L3T4^+$) lymphocytes play a significant role in the development of cerebral malaria is supported by three lines of evidence.

First, it was demonstrated that treatment of *P. berghei*-infected mice with a monoclonal antibody (MAb) directed against the L3T4 molecule completely abrogated the occurrence of cerebral malaria in these mice, although there was no modification of the infection itself (Grau et al., 1986). No protective effect was seen after treatment of infected mice with a monoclonal antibody of the same isotype directed against the $Ly.2^+$ T cell subset. The effectiveness of this treatment by anti-L3T4 MAb in infected mice was demonstrated both phenotypically (depletion of the corresponding T cell subset) and functionally (inhibition of the IgG antibody response to a T dependent antigen, tetanus toxoid).

Second, experiments were conducted in adult-thymectomized, irradiated and bone marrow-reconstituted (ATxBM) CBA mice which appeared to be completely resistant to the development of neurological lesions upon infection with *P. berghei*. These results confirmed and extended the results obtained using athymic nu/nu mice (Finley et al., 1982) which suggested a role for T cells in the development of neuropathology. T cells carrying the L3T4 phenotype were shown to be particularly involved in this syndrome since reconstitution of ATxBM CBA mice with normal $L3T4^+$ T cells rendered these mice fully susceptible to the development of neurological complications. In contrast, ATxBM mice reconstituted with the $Ly.2^+$ T cell subset appeared as protected as non-reconstituted ATxBM mice: they did not present an acute mortality associated with neurological signs, as euthymic mice did, and they died later, with severe anemia and overwhelming parasitemia (Grau et al., 1986).

Third, the exacerbation of neurological signs and the earlier mortality observed after transfer of $L3T4^+$ $Ly.2^-$ T cells from mice with cerebral malaria into euthymic mice supported the hypothesis of an involvement of these cells in the pathogenesis of cerebral malaria (Grau et al., 1986).

Humoral parameters of the immune response were also analyzed in *P. berghei*-infected CBA mice. As previously suggested (Rosenberg, 1978; Finley et al., 1982) malaria-associated polyclonal B cell activation was found to be T-cell dependent. Indeed, in infected mice treated with anti-L3T4 MAb, there was a marked inhibition of the increase in serum levels of IgG, IgM and circulating immune complexes (CIC) compared with untreated infected animals (Grau et al., 1986). The specific anti-plasmodium immune response was also studied, with particular attention to antibodies recognizing certain stages of parasite development. In fact, in malaria-infected mice treated with anti-L3T4 MAb, there was a significant decrease of the specific anti-plasmodium antibody response but this response was in no way completely suppressed. The quality of the response appeared to be more specifically influenced by the treatment with anti-L3T4 MAb: antibodies directed against mature stages of plasmodium (polysegmented schizonts) were consistently absent in the sera from those $L3T4^+$ t cell depleted mice which were protected against cerebral malaria. Moreover, anti-segmented schizont antibodies were found in only 1/15 sera from day 8-infected euthymic CBA mice without cerebral malaria. The absence of anti-segmented-schizont antibodies in the corresponding mice might be of importance in the protecting effect of anti-L3T4 MAb compared to anti-Ly.2 MAb.

Additionally, the hypergammaglobulinemia and the increase in CIC levels, absent in ATxBM mice, were restored in ATxBM mice reconstituted with normal $L3T4^+$ T cells (ATx BM.4^+). Specific antibody levels indicated that ATxBM mice responsed quite poorly to malaria antigens. Although this response was not markedly enhanced by the adoptive transfer of $L3T4^+$ T cells, it is noteworthy that this transfer lead to the appearance of anti-segmented schizont antibodies, already detectable by day 10.

The data presented here suggest that in an experimental model cerebral malaria is mediated by immune mechanisms. Indeed, it was shown that the development of murine cerebral malaria is not directly related to the degree of anemia and parasitemia, but rather appears as the expression of immunopathological reactions of the infected host. The importance of the functional integrity of lymphoid cells expressing the L3T4 phenotype (helper T cells) in the triggering

of neurological complications was outlined in these studies. One should note that L3T4$^+$ T cells of different specificities for malaria antigens may have different effects on the infection and particularly on the cerebral complications. Indeed, *in vitro*-propagated malaria-specific Ly.1$^+$ Ly.2$^-$ T cell blasts have been shown to prevent cerebral malaria upon *in vivo* transfer (Finley et al., 1983).

Various functional relationships between L3T4$^+$ T cells and neurological lesions can be envisaged. The helper effect of L3T4$^+$ T cells for the specific anti-plasmodium antibody response may be of particular importance, since it was shown that antibodies of certain specificities (segmented schizont antigens) are consistently associated with the occurrence of cerebral malaria. This helper effect may also be exerted in a less specific manner: malaria-associated polyclonal B cell activation appears indeed to be largely T-cell dependent. Secondly, the activation of T cells in the presence of properly presented malarial antigens results in the release of various lymphokines such as interleukin 2, interleukin 3, colony-stimulating factor, and -interferon. The production of -interferon was demonstrated *in vitro* by T cells in the presence of macrophages and parasitized erythrocytes (Ockenhouse et al., 1984). This was associated with the release of reactive oxygen species, participation of which has been documented in acute vascular changes (Chan et al., 1984) and suggested in the pathogenesis of cerebral malaria (Clark & Hunt, 1983). Tumor-necrosis factor (TNF) is another intermediate produced upon macrophage activation which is known to selectively alter endothelial cell functions (Nawroth et al., 1986). Thirdly, L3T4$^+$ T cells can also mediate delayed-type hypersensitivity (DTH). However, the existence of local DTH-like reactions in the cerebral compartment was not suggested by histological studies since there was no accumulation of lymphocytes at the site of brain lesions (Jerusalem et al., 1983; Depierreux et al., 1986).

The relevance of these data to human pathology should be discussed. Indeed, it was suggested that immune mechanisms are not involved in the pathogenesis of human cerebral malaria because there was no evidence, at autopsy, of cellular infiltrates nor of visible endothelial damage in brain (McPherson et al., 1985). In our experiments, there were no lymphoid cell infiltrates in the brain and the vascular lesions were acute and localized to certain cerebral territories. Therefore, the sole classical morphological analysis did not make it possible to draw conclusions regarding the existence of immunopathological mechanisms. Nevertheless, since all these lesions were prevented in mice with an impaired immune response and appeared again after restoration of the L3T4-dependent immune response, indirect mechanisms dependent on the specific or non-specific immune response to malaria are probably involved in the pathogenesis of the syndrome. One cannot exclude the possibility that similar indirect mechanisms are responsible for the cerebral syndrome in man as in our experimental model.

CONCLUSIONS AND SUMMARY

Because of the complexity of malaria parasite biology, only studies of the host immune response to the whole parasite have been so far conducted. The recently acquired knowledge of the fine molecular structure of some plasmodial antigens and their availability as synthetic or recombinant peptides represent major achievements toward the preparation of effective human malaria vaccines. They also allow for a more precise dissection of the role played by single epitopes in inducing host specific and non-specific immune response.

In this respect, our work shows that the immune response against a peptide candidate as a possible *P. falciparum* malaria vaccine, the (NANP) repetitive epitope of the CS protein, is under a strict genetic control in mice. Moreover, some data seem to indicate that this is also the case for the immune response against *P. falciparum* sporozoite in man. If this proves to be the case, then the effectiveness of such a malaria vaccine could be limited in some individuals poorly responsive to this epitope.

On the other hand, one should take into account that some complications of malaria infection can be mediated by the immune response induced by the parasite. Indeed, our work underlines that helper (L3T4$^+$) T cells, which have been shown to be specifically activated upon vaccination (Playfair et al., 1985), play a role in the induction of cerebral malaria in mice infected with *P. berghei*. It is still unclear whether this is due to a direct effect of this T cell subset or to soluble mediators released upon activation of L3T4$^+$ T cells by parasite antigens. However, it is conceivable that some particular antigens may trigger such phaenomena in poorly responsive host. Therefore, the possibility of an immunopathology induced by some antigens needs to be investigated, mainly if such antigens are being considered as potential malaria vaccines in humans.

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