Evidence for superantigenic activity during murine malaria infection

Sylviane Pied, Danielle Voegtle, Myriam Marussig, Laurent Rénia, François Miltgen, Dominique Mazier and Pierre-André Cazenave

INSERM U313, Département de Parasitologie, Groupe Hospitalier Pitié-Salpêtrière, 91, Boulevard de l'Hôpital, 75013 Paris, France
1Unité d’Immunochimie Analytique, CNRS URA 1961, Institut Pasteur, 75724 Paris Cedex 15, France
2Muséum d’Histoire Naturelle, Paris, France

Abstract

TCR Vβ usage was examined in C57BL/6 mice infected with Plasmodium yoelii. In addition to a polyclonal T cell activation, already described, a superantigenic-like activity was observed during the acute infection. This superantigenic activity induces a preferential deletion without prior expansion of CD4+ and CD8+ T cells bearing the TCR Vβ9 segment. The superantigen could be released by the parasite at different stages of its development since the deletion of Vβ9+ T cells was observed in blood and lymph nodes of mice infected either with sporozoites or with erythrocytic stages. Injection of sporozoite or parasitized erythrocytes to newborn mice led to a deletion and anergy of peripheral Vβ9+ T cells, without affecting thymic T cell populations. These observations suggest that the superantigen is released at very low concentrations during parasite development. The role of such parasite superantigenic activity in infectivity can be underlined by the observation that congenic BALB.D2 Mls1a mice lacking Vβ9 T cells are more susceptible to infection by P. yoelii.

Introduction

Malaria is still one of the most serious health problems in developing countries. The infection is contracted through the bite of an infected mosquito which releases sporozoites into the blood stream. Sporozoites invade host hepatocytes, where they differentiate giving rise to multinucleated schizonts. Mature liver schizonts release merozoites able to invade erythrocytes and initiate the erythrocytic phase of the infection. The erythrocytic stages are mainly responsible for the pathology associated with the disease (reviewed in 1).

Immunity to malaria parasites is stage-specific and develops slowly even for those living in endemic areas. This immunity is never complete, and is reflected by reduced clinical symptoms and low parasitemia. The immunology of the disease is far from being fully understood, but it is clear that αβ T cells and B cells are important in regulating and maintaining immune responses to parasite exo-erythrocytic and erythrocytic stages (2,3). The evidence of a role for T cell immunity in controlling malaria infection has been derived from both animal and human models (4–13). T cells have been implicated to a great extent in the pathology of the disease (14,15), and cytokines such as tumor necrosis factor and IFN-γ have also been shown to play a critical role in resistance to infection (16–18).

Profound perturbations of the immune system are observed during the infection such as (i) hypergammaglobulinemia, with a lack of plasmodial specificity resulting from a polyclonal B cell activation (19,20), and (ii) induction of hyporesponsiveness of T cells to plasmodial and non-plasmodial antigens, and perturbation of the CD4/CD8 ratio (21–23). The cause of this phenomenon is not known, nevertheless there is evidence of a defect in both production of IL-2 and expression of IL-2 receptor by peripheral blood lymphocytes in response to stimulation with malaria-specific antigen during acute Plasmodium falciparum malaria in humans (24).

It has been suggested that the strong polyclonal activation of all lymphocyte populations observed during malaria infection is due to a mitogen released by Plasmodium (25). To date, no evidence has been provided concerning the existence of such a mitogenic molecule in human or rodent Plasmodium species. Erythrocytic stages of P. falciparum parasites have been shown to be able to stimulate in vitro proliferation of γδ T cells from non-exposed donors, and especially those T cells using Vδ9 and Vδ2 (26,27). Since only Vδ9+ T cells were stimulated it was suggested that the γδ T cell response to P. falciparum was due to a superantigen since it induced a response MHC class II molecules. The in vitro observation...
was underlined by the fact that the V$_{δ}$T cell subset increased in the blood of individuals undergoing primary *P. falciparum* infection (28,29).

In general, superantigens from a number of pathogens stimulate T lymphocytes through particular TCR V$_{δ}$ chains (reviewed in 30). The recognition of T cells depends almost exclusively on the V$_{δ}$ domain and, consequently, a superantigen can interact with a large fraction of the T cell repertoire, because the number of V$_{δ}$ genes is low (31). In contrast to conventional antigens, superantigens bind specifically with a region of TCR located on the V$_{γ}$ chain, outside of the specific site which combines with the MHC–peptide complex (32,33). When superantigens are encountered during T cell development they usually induce a clonal deletion, or anergy, of T cells bearing the appropriate V$_{γ}$ (34).

In order to identify T cell populations and define the T cell repertoire involved in the host response to malaria infection, we have studied TCR V$_{γ}$ chain usage in different cell compartments of C57BL/6 mice infected either with sporozoites or erythrocytic stages of *Plasmodium*. We describe here a superantigenic-like activity associated with the parasite.

**Methods**

**Mice**

C57BL/6 and BALB/c mice were obtained from Charles River (St-Aubin les Elbeuf, France); congenic BALB.D2 Mls$^*$ were bred at the Institut Pasteur from a breeding pair kindly given by Dr Martine Bruley-Rosset (INSERM U267, Villejuif, France).

**Parasite and infection**

265 BY strain of *P. yoelii yoelii* maintained as described previously (35) was used for these experiments. Sporozoites were obtained from infected salivary glands of *Anopheles stephensi* mosquitoes, 16–21 days after an infective blood meal. After aseptic dissection, salivary glands were homogenized in a glass grinder and diluted in sterile PBS. Mice were infected either by i.v. injection of 4000 sporozoites of the 265 BY *P. yoelii* strain or i.p injection of $1 \times 10^5$ parasitized red blood cells (pRBC) of *P. yoelii*. In neonatal deletion experiments, 2-day-old mice were injected i.p with 50 µl of 4000 sporozoites or $1 \times 10^6$ pRBC suspensions. Control animals were infected with sterile PBS. Parasitemia was monitored by detecting parasites every day on blood smears after Giemsa staining.

**Antibodies**

mAb specific for mouse CD3 (hamster anti-CD3 mAb 2C11), CD4 (rat anti-CD4 mAb GK1.5, gift from M. Pierres, Centre d’Immunologie, Marseille-Luminy, France) and CD8 (mouse mAb in a final volume of 200 µl) were injected either with live or sporozoites lysate or pRBC in 0.2 ml of RPMI 1640 (Life Technologies, Cergy-Pontoise, France) containing 10% FCS, L-glutamine, 5×10$^{-5}$ M 2-mercaptoethanol and antibiotics per well were stimulated in triplicate with concanavalin A (1 µg/ml) or with different immobilized anti-V$_{δ}$ chains (10 µg/ml): anti-CD3 (145-2C11), anti-V$_{γ}8$ (KJ16) and anti-V$_{γ}9$ (MR10.2) at 37°C (95% humidity and 5% CO$_2$) in 96-well round-bottomed plastic micro-culture plates (Costar). $[^{3}H]$Thymidine (1 µCi; Amersham, Les Ulis, France) was added per well 18 h before harvesting cells. Thymidine incorporation was determined by beta counting (Beckman, Gagny, France).

**Lymphocyte preparations and staining**

Blood, lymph nodes (LN) and spleens were removed from control uninfected mice and mice infected with sporozoites 3, 10 and 30 days before injection. In the case of mice injected with pRBC, organs were taken 7 and 30 days post-infection. LN cells and splenocytes suspensions were treated twice with ACK (ammonium chloride/potassium) buffer to lyse erythrocytes and washed twice with PBS/FCS before counting. For cytofluorometric analysis, lymphocytes were incubated first with biotinylated mAb directed against the V$_{γ}$ chain and subsequently with anti-CD3–FITC, anti-CD4–FITC or anti-CD8–FITC in the presence of phycoerythrin-conjugated streptavidin.

**Flow cytometric analysis**

Cell analysis was done with a FACScan cytometer (Becton Dickinson, Grenoble, France) using Lysis II software. Viable lymphocytes were carefully gated by light scatter (FSC/SSC), and for all V$_{γ}$ analysis 5000–10,000 events were acquired and recorded per sample. The percentage of fluorescent positive cells was determined by integrating profiles determined on the basis of viable lymphocytes.

**Lymphocyte cultures and proliferation tests**

To analyze peripheral stimulation of T cells by malarial proteins, 8-week-old female C57BL/6 were injected in each footpad with 50 µl of solution containing either PBS or homogenate corresponding to 4000 lysed sporozoites or $1 \times 10^6$ lysed pRBC. Popliteal LN were taken 10 days after injection and lymphocytes were isolated. For assessment of in vitro T cell proliferation, $2 \times 10^5$ LN cells from newborn mice infected either with live or sporozoites lysate or pRBC in 0.2 ml of RPMI 1640 (Life Technologies, Cergy-Pontoise, France) containing 10% FCS, L-glutamine, 5×10$^{-5}$ M 2-mercaptoethanol and antibiotics per well were stimulated in triplicate for 5 days with concanavalin A (1 µg/ml) or with different immobilized anti-V$_{δ}$ chains (10 µg/ml): anti-CD3 (145-2C11), anti-V$_{γ}8$ (KJ16) and anti-V$_{γ}9$ (MR10.2) at 37°C (95% humidity and 5% CO$_2$) in 96-well round-bottomed plastic micro-culture plates (Costar). $[^{3}H]$Thymidine (1 µCi; Amersham, Les Ulis, France) was added per well 18 h before harvesting cells. Thymidine incorporation was determined by beta counting (Beckman, Gagny, France).

**IL-2 production assay**

IL-2 production of T cells from mice treated with various stimuli was tested using an IL-2-dependent T cell line (CTLL-2) stimulated with medium alone, or supernatant from cells previously stimulated with concanavalin A or different anti-V$_{δ}$ mAb in a final volume of 200 µl 10% FCS RPMI in microtiter plates. IL-2 was quantitated using the standard assay of $[^{3}H]$thymidine incorporation by the IL-2-dependent CTLL cell line.

**Statistical analysis**

Differences between groups were analyzed by Student’s $t$-test and $χ^2$ using the STAVIEW software.
infected erythrocytes in the βLN compartment, the number of CD3 lymphocytes is slightly

\[ \text{TCR V}^\beta \text{ repertoire of CD3 T cells during infection by } P. \text{ yoelii} \]

The distribution of lymphocyte subpopulations was examined by cytofluorometry in different cell compartments of C57BL/6 mice infected either with sporozoites or pRBC of P. yoelii 265 BY. Numbers of CD3\(^+\), CD4\(^+\) and CD8\(^+\) T cells were determined for blood, LN and spleen at days 3, 10 and 30 after infection with sporozoites, and days 7 and 30 after parasitized erythrocyte inoculation. A significant change was observed in the number of CD3\(^+\) T cells in peripheral blood lymphocytes at day 10, in the group of mice infected with sporozoites, and at day 7 after pRBC inoculation. This increase affects both CD4\(^+\) and CD8\(^+\) T cell subsets (Table 1). In the LN compartment, the number of CD3 lymphocytes is slightly affected during the acute phase of the parasitemia (day 10 in mice infected with sporozoites and day 7 in those inoculated with parasitized erythrocytes) (Table 2). The principal modification seen was a net increase of CD4\(^+\) T cells (1.6 times more than controls in sporozoite-inoculated mice and three times more in mice inoculated with pRBC). The number of CD8\(^+\) T cells was also affected by the parasite development, but only in the group of mice which received pRBC. The increase of CD3\(^+\) T cells and particularly by the CD4\(^+\) subpopulation observed during the parasitemia is maintained in the group of mice infected with pRBC, even after mice recovered from the infection. In the spleen of mice inoculated either with sporozoites or pRBC, the infection was also associated with an increase of CD4\(^+\) T cells with time, even after remission (Table 3). In parallel, a significant fall in the number of T cells bearing the CD8 marker was observed, particularly in the group of mice inoculated with sporozoites. This drop in CD8\(^+\) T cells was maintained, even 30 days after parasite infection.

\[ V^\beta \text{ TCR repertoire of CD3 T cells during infection by } P. \text{ yoelii} \]

In order to determine whether the TCR V\(^\beta\) gene usage was disturbed by infection with malaria parasites, the in vivo responses were assessed by enumerating T cells using different V\(^\beta\) in both LN and blood at various times after parasite inoculation. We have observed a slight increase of V\(^\beta\)-bearing T cells in blood of mice injected with pRBC at day 10 and 30, and in LN of mice infected with sporozoites at day 3 (Fig. 1).
Table 3. Phenotype of splenic T cells from C57BL/6 mice infected with *P. yoelii*

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>Total no. of cells (×10^6)</th>
<th>Percentage of cells (absolute no. ×10^6)</th>
<th>CD3&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CD4&lt;sup&gt;b&lt;/sup&gt;</th>
<th>CD8&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CD4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>CD8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sporozoites</td>
<td></td>
<td></td>
<td>43.9 ± 2.3</td>
<td>20.10 ± 4.4</td>
<td>16.3 ± 1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>46.6 ± 2.5</td>
<td>16.10 ± 1.9</td>
<td>18.6 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>36.4 ± 4.8(10.3 ± 2.9)</td>
<td>13.70 ± 4.2(3.89 ± 1.1)</td>
<td>3.9 ± 1.1(1.1 ± 0.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>33.5 ± 1.9</td>
<td>18.60 ± 0.75</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected erythrocytes</td>
<td></td>
<td></td>
<td>28.6 ± 3.3</td>
<td>10.04 ± 5.1</td>
<td>6.7 ± 1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>37.8 ± 5.9(10.8 ± 3.0)</td>
<td>18.3 ± 4.9(2.8 ± 1.3)</td>
<td>5.2 ± 1.1(2.8 ± 1.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± SD of cell counts recovered from five mice in each group.

<sup>b</sup>Percentage and absolute numbers (in parentheses) of total cells determined after staining with FITC-labeled anti-CD3 antibody.

<sup>c</sup>Percentage and absolute numbers (in parentheses) of each population (CD4<sup>+</sup> and CD8<sup>+</sup>) in CD3<sup>+</sup> T cells.

---

**Fig. 1.** TCR V<sub>β</sub> usage in peripheral blood lymphocytes and LN cells from *P. yoelii*-infected mice. The distribution of the indicated V<sub>β</sub> TCR segments on CD3<sup>+</sup> T cells from blood and LN of C57BL/6 infected mice were determined by two-color FACS analysis at days 3, 10 or 30 after parasite inoculation. Bar chart represents the mean percentage ± SD of five mice studied individually. Control mice are shown with clear bars and infected mice with hatched bars.

---

are in progress and will be reported later. Moreover, as shown in Fig. 1, even as soon as day 3 of infection with *P. yoelii* sporozoites, the frequency of CD3<sup>+</sup> V<sub>β</sub>9-bearing T cells was significantly decreased in the LN. This CD3<sup>+</sup> V<sub>β</sub>9<sup>+</sup> T cell decrease was also observed at day 10 in both lymphoid compartments tested in mice infected either with sporozoites or pRBC, as compared with control mice. Thirty days after parasite inoculation, when mice had recovered from the disease, expression of CD3<sup>+</sup> V<sub>β</sub>9<sup>+</sup>-bearing T cells was restored in both the blood and LN of parasitized mice at a level comparable to controls. These results suggested the occurrence of a superantigenic activity associated with the pres-
Superantigen-like effect in malaria parasites

Fig. 3. Lysates of *P. yoelii* sporozoites or pRBC do not induce expansion of Vβ9 CD3⁺ T cells. Either 50 µl of 4000 lysed sporozoites (experiment A) or 10⁶ lysed parasitized erythrocytes: (experiment B) were administered to mice footpads. Ten days later the expression of Vβ7, 8 and 9⁺ T cells in the popliteal LN was determined by FACS analysis. The data presented are expressed as mean percentage of the result obtained from five mice of each group (□) control and (■) treated.

Fig. 2. Vβ9 representation among CD4⁺ and CD8⁺ in peripheral blood lymphocytes and LN cells from *P. yoelii*-infected mice. Vβ9 expression among CD4⁺ and CD8⁺ T cells of blood and LN from control mice (□) and mice infected either with sporozoites (■) or erythrocytic stages (■) were compared at day 10 after inoculation. The results are expressed as percentage of positive cells in each experimental group analyzed by FACS. Each bar correspond to one group. Five mice were tested per group.

ence of *P. yoelii* parasites. It is worthy to note that the deletion of Vβ9-bearing T cells was observed in the LN very soon after parasite inoculation (day 3), and that there was a close relationship between the deletion at the periphery and the presence in blood of the replicating parasite during which specific malaria antigens are released.

To determine the population of blood and LN T cells affected by the Vβ9 deletion, we have analyzed the frequency of expression of the Vβ9 chain among CD4⁺ and CD8⁺ T lymphocytes at day 10 which corresponds to the peak of parasitemia in infected mice. The results of this analysis are shown in Fig. 2. The deletion of Vβ9-bearing T cells affects both CD4⁺ and CD8⁺ T subsets in mice infected either with sporozoites or with erythrocytic stages as compared to the control group.

*T cell response to sporozoites and pRBC antigens*

In order to analyze the mechanisms involved in the Vβ9 deletion, mice were injected in their footpads with either lysed sporozoites or lysed pRBC homogenates emulsified in complete Freund’s adjuvant and 10 days later lymphocytes were isolated from popliteal LN. Proliferation of T cells was observed following the *in vivo* priming with both types of antigen preparation when compared to control mice which had received complete Freund's adjuvant alone. In each group, we have examined the level of expression of a number of Vβ chains including Vβ9. As shown in Fig. 3, the percentages of T cells expressing Vβ7, 8 or 9 were similar in mice primed with either parasite antigen. These results suggested that the expression by *P. yoelii* of a Vβ9-specific superantigenic activity is an active phenomenon which requires live parasites.

**Vβ9 deletion in mice neonatally infected with *P. yoelii***

To confirm that the deletion of Vβ9-bearing T cells resulted from superantigenic activity associated with the *P. yoelii* infection, we analyzed the expression of different Vβ chains (including Vβ9) in C57BL/6 mice injected 48 h after birth with either developmental stage of live parasites (sporozoite or pRBC). In parallel, a group of mice of the same age received an injection of sporozoite lysate (the number of sporozoites present in the lysate being the same as the number of live sporozoites used to infect the mice in the first group). Four weeks later the expression of Vβ9 in the thymus and peripheral blood lymphocytes of mice belonging to the different groups was analyzed. As shown in Fig. 4, a deletion of Vβ9⁺ T cells was observed in the peripheral blood lymphocytes of neonatal mice parasitized with either sporozoites or pRBC, but not in mice that received just lysed parasites. By contrast, no Vβ9⁺ deletion was observed for peripheral T cells other than Vβ9⁺, as is exemplified in Fig. 4 by Vβ14. It is noteworthy that a Vβ9 deletion in the thymus was not observed.

In parallel, we examined the proliferative capacity of mesenteric LN T cells from control and mice neonatally infected either with lysed or live sporozoites, or with pRBC in the presence of concanavalin A or anti-CD3, anti-Vβ9 or anti-Vβ9 mAb coated on the surface of culture plates. Data represented...
Fig. 5. Effect of an in vitro stimulation by anti-Vβ9 of LN cells sensitized in vivo by the parasite. Parasites were administered i.p. to mice 48 h after birth. Four weeks later, the mice were killed and T cells from LN recovered. Proliferation of cells induced by the different stimuli was assessed by thymidine incorporation measured at day 5. The result is presented as the mean c.p.m. number of triplicate cultures.

Discussion

In this report, we have examined TCR Vβ usage during a murine experimental malaria infection. Using two-color flow cytometry, we have assessed the percentage of cells utilizing the different Vβ families in peripheral blood lymphocytes and LN of C57BL/6 mice infected either with sporozoites or with erythrocytic stages of P. yoelii at 3, 10 and 30 days after parasite inoculation. The results obtained are consistent with a parasite superantigenic like-activity during the acute malaria infection. By day 3 after inoculation, mice which had been exposed to sporozoites showed a preferential deletion of a Vβ9-specific superantigenic activity associated with infection by P. yoelii. They also confirm that this activity is only detectable when the parasite is alive.

Course of parasitemia in Vβ9-deleted mice

We have addressed the question of the direct or indirect interference of Vβ9-bearing T cells in the susceptibility to infection by P. yoelii. For this purpose, in a first step, we have verified in the BALB/c strain that the Vβ9+ T lymphocytes were indeed deleted during the infection by the parasite (data not shown). Next, we have taken the course of parasitemia in BALB/c mice as a control and compared it to parasitemia observed in the BALB.D2 Mlsa mice, a congenic strain which bears an integrated Mtv provirus (Mtv7) able to delete, Vβ6, Vβ8.1 and Vβ9 positive T cells (51). As shown in Fig. 6, the infection by 4000 sporozoites of P. yoelii was self limiting in both strains. However, BALB.D2 mice lacking Vβ9 were more susceptible to the infection than BALB/c, since the peak of parasitemia and its duration were greater in these mice (mean peak of parasitemia was 15% in BALB.D2 between days 9 and 11 compared to 7.5% in BALB/c around day 8–9).
Superantigen-like effect in malaria parasites

Fig. 6. Course of P. yoelii infection in congenic BALB.D2Mls\(^a\) mice. Groups of BALB/c (n = 6) and BALB.D2 Mls/a (n = 10) were inoculated i.v. with 4000 sporozoites of P. yoelii. Mice are plotted individually. Parasitemia was checked every day.

expansion of the target T cells bearing the appropriate V\(_{\beta}\)(s), before the clonal deletion or anergy of this target subset (53). By contrast, the deletion of V\(_{\beta}9\)-bearing T lymphocytes induced by a molecule associated with P. yoelii infection is not preceded by any detectable T cell expansion. This observation is reminiscent of the detailed work on the in vivo expression of different V\(_{\beta}\) in response to a superantigen (staphylococcal enterotoxin A) (54). It was observed that repeated injections of very small doses of the superantigen caused almost complete deletion of the T cells bearing the target V\(_{\beta}\), with the absence of any prior T cell expansion. By analogy, we suggest that the plasmodial superantigenic activity probably acts also at a very low concentration and is released by the parasite at different stages of its development, since its activity is detectable after inoculation of either sporozoites or pRBC. Alternatively, it is possible that the parasite has an effect on host cells, by an unknown mechanism, in such a way that some of these cells are directly or indirectly activated to synthesize, at low concentration, an ‘endogenous’ superantigen.

The in vivo response to bacterial toxins usually results in a preferential deletion, or anergy, in the responding CD4\(^+\) subpopulation; although some exceptions have been observed where both CD4\(^+\) and CD8\(^+\) subsets are affected (55). The infection with exogenous MMTV is dominated by deletion or anergy in the responding CD4\(^+\) lymphocytes (56). In the murine model of Chagas’ disease, the in vivo Trypanosoma cruzi superantigenic effect was observed in the CD8 compartment (57). In a second parasitic system which concerns Toxoplasma gondii, the in vitro response to a superantigen is restricted to CD8\(^+\) lymphocytes (58). By contrast, the P. yoelii superantigenic activity affects both V\(_{\beta}9\)-CD4\(^+\) and CD8\(^+\) subsets. Again, by analogy with the effects obtained in the chronic exposure to low dose of SEA (discussed above) which results in the disappearance of CD4\(^+\) and CD8\(^+\) T cells bearing V\(_{\beta}3\), we can hypothesize that the plasmodial superantigen is expressed at low levels during the time of the infection.

In order to confirm the superantigenic activity observed during malaria infection, sporozoites or erythrocytic stages of P. yoelii were injected into newborn C57BL/6 mice. Four weeks later we observed deletion and anergy of peripheral V\(_{\beta}9\) \(1\) T cells, with no effect detectable in the thymus. In general, neonatal injection of bacterial or viral superantigen induces a V\(_{\beta}\)-specific clonal deletion of T cells expressing the reactive V\(_{\beta}\) domain during thymic maturation, as well as at the periphery. However, by the use of several congenic strains, carrying the MMTV (C3H) superantigen as a transgene and caused almost complete deletion of the T cells bearing the target V\(_{\beta}\), with the absence of any prior T cell expansion. By analogy, we suggest that the plasmodial superantigenic activity probably acts also at a very low concentration and is released by the parasite at different stages of its development, since its activity is detectable after inoculation of either sporozoites or pRBC. Alternatively, it is possible that the parasite has an effect on host cells, by an unknown mechanism, in such a way that some of these cells are directly or indirectly activated to synthesize, at low concentration, an ‘endogenous’ superantigen.

The in vivo response to bacterial toxins usually results in a preferential deletion, or anergy, in the responding CD4\(^+\) subpopulation; although some exceptions have been observed where both CD4\(^+\) and CD8\(^+\) subsets are affected (55). The infection with exogenous MMTV is dominated by deletion or anergy in the responding CD4\(^+\) lymphocytes (56). In the murine model of Chagas’ disease, the in vivo Trypanosoma cruzi superantigenic effect was observed in the CD8 compartment (57). In a second parasitic system which concerns Toxoplasma gondii, the in vitro response to a superantigen is restricted to CD8\(^+\) lymphocytes (58). By contrast, the P. yoelii superantigenic activity affects both V\(_{\beta}9\)-CD4\(^+\) and CD8\(^+\) subsets. Again, by analogy with the effects obtained in the chronic exposure to low dose of SEA (discussed above) which results in the disappearance of CD4\(^+\) and CD8\(^+\) T cells bearing V\(_{\beta}3\), we can hypothesize that the plasmodial superantigen is expressed at low levels during the time of the infection.

In order to confirm the superantigenic activity observed during malaria infection, sporozoites or erythrocytic stages of P. yoelii were injected into newborn C57BL/6 mice. Four weeks later we observed deletion and anergy of peripheral V\(_{\beta}9\) \(1\) T cells, with no effect detectable in the thymus. In general, neonatal injection of bacterial or viral superantigen induces a V\(_{\beta}\)-specific clonal deletion of T cells expressing the reactive V\(_{\beta}\) domain during thymic maturation, as well as at the periphery. However, by the use of several congenic strains, carrying the MMTV (C3H) superantigen as a transgene and expressing it at different levels, it was recently shown that a low level of superantigen transgene expression has no effect on the target V\(_{\beta}14\) \(1\) T cells in the thymus, although the superantigen is expressed sufficiently to delete V\(_{\beta}14\) \(1\) T cells in the periphery (59).

Our results showing the occurrence of superantigenic activity during the infection by P. yoelii raises the question of its role in the infectivity of the parasite, or in the pathogenesis of the infection, or in both. Therefore, we have analyzed the course of infection in BALB.D2 mice, a BALB/c congenic for Mls\(^a\) which deletes V\(_{\beta}9\). When compared to BALB/c, the infectivity of the P. yoelii is increased in BALB.D2 mice. This result can be compared with data obtained in the same strains of mice with a viral superantigen. The nucleocapsid of the rabies virus has been shown to delete the CD4\(^+\) V\(_{\beta}6\)-bearing T cells and it was observed that BALB.D2 (which also presents a deletion for V\(_{\beta}6\)) is less susceptible than the BALB/c strain to the pathogenesis of the central nervous system (60). Since the superantigenic activity of the rabies virus was not associated with live intact virus, in contrast to our observations in malaria where live parasites were necessary, the comparison between the two cannot be taken further.

The system that we have described in this paper offers a good model to study the potential role of superantigens during primary malaria infection in correlation with susceptibility. Superantigens may play a role in host susceptibility to the establishment of a chronic infection after a single inoculation.
and also to reinfection of the same parasite. Several experiments are in progress to clarify this important issue.

Acknowledgements

We would like to thank Drs Gordon Langsley and Antonio Coutinho for fruitful discussions and advice, Monique Bauzou for parasite detection on blood smears, Mathias Faure for IL-2 quantification, and Michèle Berson for her help in preparing the manuscript. This research was supported a grant from CNAMTS no. 4AP101.

Abbreviations

LN lymph node
pRBC parasitized red blood cells

References

Superantigen-like effect in malaria parasites


