Malaria-Specific and Nonspecific Activation of CD8+ T Cells during Blood Stage of Plasmodium berghei Infection

Mana Miyakoda, Daisuke Kimura, Masao Yuda, Yasuo Chinzei, Yoshisada Shibata, Kiri Honma and Katsuyuki Yui

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Malaria remains one of the crucial threats to public health in much of the world. It has been well accepted that Ab and CD4^+ T cells play critical roles for protection against malaria parasites that can be acquired during natural or experimental infection (1–4). However, the role of CD8^+ T cells in protective immunity is controversial. Some studies suggested that CD8^+ T cells could transfer protective immunity to an adoptive host (5), whereas others claimed that they did not play a major role in protection against blood stage infection with Plasmodium species (6). In contrast, accumulating evidence indicates that CD8^+ T cells are involved in the pathogenesis of severe malaria. Cerebral malaria resulting from Plasmodium falciparum infection is one of the most severe complications and main cause of death in human malaria (7–11). Using a rodent model of malaria infection with the Plasmodium berghei ANKA (PbA) strain, investigations indicated that CD8^+ T cells are one of the major effectors that trigger cerebral malaria. In these experimental models, it was shown that CD8^+ T cells were sequestered in the brain during cerebral malaria and that the depletion of CD8^+ T cells decreased mortality (8). Furthermore, perforin-mediated killing by CD8^+ T cells was required for the pathogenesis of experimental cerebral malaria resulting from PbA infection, suggesting that the effector function of CD8^+ T cells is involved in the pathogenesis (9). However, it is unclear whether Plasmodium-specific CD8^+ T cells are activated during the erythrocyte stage of malaria and how they are involved in the pathogenesis of cerebral malaria.

Because malaria parasites infect RBC that do not themselves express MHC molecules, the infected cells cannot directly present malaria Ags to T cells in association with MHC class I molecules. To activate specific CD8^+ T cells, malaria Ags must be presented by a process referred to as cross-presentation by APCs that are themselves not infected, as reported for some minor histocompatibility Ags, tumor Ags, and various pathogens (12, 13). There are two main Ag presentation pathways of cross-presentation; one is the phagosome-to-cytosol pathway that is dependent on the TAP molecule (14), and the other is the TAP-independent pathway in which antigenic peptide is generated and loaded to MHC class I in MHC class II compartments (15) or on the cell surface by peptide regurgitation (16). The former TAP-dependent pathway is used by viruses that do not themselves infect hematopoietic cells (17) or some of the intracellular parasites such as Mycobacterium tuberculosis and Toxoplasma gondii (18, 19). The latter TAP-independent pathway is used by some pathogen-derived Ags such as those from Leishmania major and virus-like particles (20, 21). It is unclear how these different pathways are selected in targeting the exogenous Ags to the MHC class I presentation pathway by each microbial species.

Because class I-restricted Ags have not been identified in malaria parasites during the erythrocyte stage, we used a model Ag OVA to study immune responses of Ag-specific CD8^+ T cells...
during malaria infection. We generated a recombinant PbA that expresses a cytoplasmic form of OVA (OVA-PbA). Using this system, we show that malaria Ags can be presented to specific CD8\(^+\) T cells by cross-presentation in a TAP-dependent manner during the erythrocyte stage of malaria infection and that these activated CD8\(^+\) T cells could be pathogenic to the host. Furthermore, CD8\(^+\) T cells that are not specific for malaria Ag can be activated during malaria infection in an Ag-nonspecific manner. This activation was at least in part dependent upon the presence of NK cells.

Materials and Methods

Generation of recombinant PbA parasite clones expressing OVA

Recombinant PbA parasites were engineered to constitutively express a truncated C-terminal fragment of OVA (aa 150–386) fused to the N-terminal sequence (aa 1–5) of the PbA heat shock protein (hsp) 70 gene. The gene construct was based on PbU-scripts KS\(^+\) (Stratagene) and contains the following elements: 1) PbA dihydrofolate reductase-thymidylate transferase-ts DHFR-ts gene; 2) PbA hsp70 5'-untranslated region and N-terminal coding sequence; 3) coding sequence of C-terminal fragment of OVA; 4) PbA hsp70 3'-untranslated region and DHFR-ts 3'-untranslated region. The DHFR-ts gene contains a point mutation at position 110 of the DHFR gene causing a Ser\rightarrow Asn transition conferring resistance to the antimalarial drug pyrimethamine.

The procedure to generate recombinant PbA was described previously (22). In brief, the gene construct was digested with SacI and KpnI to linearize and release the insert from the vector. PbA merozoites were transfected by electroporation and were selected in rats using pyrimethamine. The surviving parasites were further selected by limiting dilution in mice, and parasite clones that were resistant to pyrimethamine were obtained.

Mice, adoptive transfer, and PbA infection

OT-I-transgenic mice expressing the TCR specific for OVA\(_{257-264}\)/K\(_b\) (23), were provided by Dr. H. Kosaka (Osaka University, Osaka, Japan), TAP knockout (TAP-KO) mice (C57BL/6 (B6) background; Ref. 24) by Dr. H. Watanabe (University of the Ryukyus Okinawa, Japan), B6.SJL-Ptprc congenic (B6.SJL) mice (CD45.1\(^+\)) by Dr. Y. Takahama (University of Tokushima, Tokushima, Japan), and RAG2 knockout (RAG2-KO) mice (25) by Dr. Y. Yoshikai (Kyushu University, Fukuoka, Japan). TCR\(_{P14}\) lymphocytic choriomeningitis virus (LCMV)/TCR\(_{P14}\)-KO mice (26) were purchased from Taconic. B6 mice were purchased from SLT, OT-I and B6.SJL mice were bred, and offspring were intercrossed to obtain CD45.1 OT-I mice. RAG2-KO mice and OT-I mice were intercrossed to obtain RAG2-KO OT-I mice. These mice were maintained in the Laboratory Animal Center for Animal Research at Nagasaki University (Nagasaki, Japan) and were used at the age of 8–14 wk. For adoptive transfer, CD8\(^+\) T cells (>95%) were purified from CD45.1 OT-I mice using anti-CD8 IMag (BD Biosciences), labeled with CFSE (15\(^\mu\)M; Molecular Probes) and were injected into the tail vein of B6 mice (0.7–2 \times 10\(^5\)/mouse). Mice were infected with WT-PbA or OVA-PbA by i.p. injection of parasitized RBCs (10\(^5\) infected RBCs or 10\(^6\) infected RBCs) or those infected with WT-PbA or OVA-PbA for 2 days, and pulsed for 20 h with [\(^{3}H\)Tdr]. Paraisisma levels: WT-PbA, 16.9%; OVA-PbA, 18.4%. D, OT-I spleen cells (1 \times 10\(^5\)) were cultured in the presence of DCs (CD11c\(^+\) cells, 3 \times 10\(^5\)) and RBCs from uninfected mice or mice infected with WT-PbA (\(\bullet\)) or OVA-PbA (\(\bigcirc\)) for 48 h. The production of IFN-\(\gamma\) in the supernatant was determined by ELISA.

Proliferation and IFN-\(\gamma\) production in vitro

To isolate dendritic cells (DC), B6 spleen was treated with dispase (5 \(\mu\)g/ml; Goto-shusei) for 30 min at 37°C. Spleen cells were treated with anti-CD11c microbeads, and DCs (CD11c\(^+\)) were isolated from the culture of OT-I CD8\(^+\) T cells (1 \times 10\(^5\)) in the presence of DC (3 \times 10\(^5\)) and crude RBC Ag (9.3 \times 10\(^5\) RBCs) for 63 h with [\(^{3}H\)Tdr]. For IFN-\(\gamma\) assay, OT-I CD8\(^+\) T cells (3 \times 10\(^5\)) were stimulated with DCs (1 \times 10\(^5\)) and crude RBC Ag (9.3 \times 10\(^5\) RBCs) for 48 h, and the levels of IFN-\(\gamma\) in the supernatant were determined by a sandwich ELISA as described previously (28).

Flow cytometry

FcR was blocked with anti-FcγRI/III mAb (BD Biosciences). The staining reagents used in this study include PE-Cy7-anti-CD45.1, allophycocyanin- or PE-anti-CD8, FITC-anti-CD4, PE- or FITC-anti-CD62 ligand (CD62L), FITC- or biotin-anti-CD44, and PE- or FITC-anti-CD69 mAbs (eBiosciences). OVA\(_{257-264}\)-H-2\(K\(_b\) tetramer was purchased from MBL. 7-Aminoactinomycin D was added to exclude dead cells from the analysis. For analysis of granzyne B expression, splenocytes were incubated with Fc block and were stained with PE-Cy7-anti-CD45.1 and FITC- or allophycocyanin-anti-CD6 mAbs. Samples were fixed and permeabilized using Cytofix-Cyperm buffer (BD Biosciences), stained with PE-anti-granzyme B mAb (Caluag), and analyzed using FACScanCanto II (BD Biosciences). Cytotoxicity assay in vitro and in vivo

For in vitro cytotoxicity assay, CD8\(^+\) T cells were enriched from spleen cells by CD8a\(^+\) T cell isolation kit (Miltenyi Biotech). EL4 target cells
Percent specific lysis was determined by the presence of the typical pathological signs as described (29):

Evaluation of the disease

Mice were monitored daily after day 5 of infection, and clinical scores were defined by the presence of the typical pathological signs as described (29):

Clinical scores were analyzed using flow cytometry. Levels of parasitemia: WT-PbA, 1.3%; OVA-PbA, 1.0%.

A six independent experiments similar to those described were used for the calculations.

Statistical analysis

In comparison of three or more groups, overall comparison was first made by ANOVA for one-way or two-way data at the significance level of 0.05, and if significant, each pair of the groups was compared by t test. If the ordinary ANOVA was considered to be inappropriate because of the significant departure from normality, censoring in measurements or large variation in the variance among groups, the Kruskal-Wallis test, the Wilcoxon rank-sum test, or the Savage test was used instead with the significance level determined by the Bonferroni procedure controlling the familywise error rate <0.05. For survival data, the log-rank test was used in a similar way. Procedures of ANOVA, LIFETEST, and NPAR1WAY in the SAS system were used for the calculations.
Results
Ag-specific and nonspecific activation of CD8$^+$ T cells during PbA infection

We generated recombinant malaria PbA parasite that constitutively expresses the C-terminal fragment of OVA (aa 150–386) fused to the N terminus of PbA hsp70 (OVA-PbA; Fig. 1A). The expression of the recombinant OVA was confirmed by immunoblotting of the infected RBC lysates with anti-OVA Ab (Fig. 1B). The ability of DCs to present OVA-PbA Ag was evaluated for proliferation and IFN-γ secretion of OVA-specific OT-I CD8$^+$ T cells in vitro (Fig. 1, C and D). CD8$^+$ T cells from OT-I mice showed specific proliferation and IFN-γ secretion in response to the DCs pulsed with OVA-PbA-infected RBC. We used adoptive transfer of OT-I CD8$^+$ T cells from TCR-transgenic mice to determine whether Ag-specific CD8$^+$ T cells can be primed during PbA infection. B6 mice (CD45.2$^+$) were adoptively transferred with CFSE-labeled OT-I CD8$^+$ T cells (CD45.1$^+$) and were infected with wild-type P. berghei ANKA (WT-PbA) or OVA-PbA. The proliferation of OT-I CD8$^+$ T cells was monitored by the sequential loss of CFSE intensity 7 days after infection (Fig. 2, A and B). The proportion of OT-I CD8$^+$ T cells increased in mice infected with OVA-PbA (19.4%) when compared with uninfected mice (4.2%) or mice infected with WT-PbA (3.1%). OT-I CD8$^+$ T cells divided minimally and remained CD69$^{low}$CD44$^{hi}$CD62L$^{hi}$ in the uninfected host. Extensive division of OT-I CD8$^+$ T cells was observed in mice infected with OVA-PbA. In addition, up-regulation of the CD69 marker was observed in cells that divided 0–3 times, and up-regulation of CD44 and down-regulation of CD62L were evident in cells that have divided multiple times, indicating that OT-I CD8$^+$ T cells were activated in an Ag-specific manner during infection with PbA. Unexpectedly, OT-I CD8$^+$ T cells divided ~4 times and up-regulated the CD69 marker in mice infected with WT-PbA, suggesting that OT-I CD8$^+$ T cells can be activated in an Ag-nonspecific manner during infection with WT-PbA. Because it was recently reported that malaria infection impairs cross-presentation (30), we examined the ability of host APC to present OVA-PbA Ag during early (days 0–3) and late (days 5–8) periods after infection (Figs. 2C and 4A, left). In both periods, OT-I cells proliferated several times in vivo after infection with OVA-PbA but not with WT-PbA, indicating that APC can cross-present malaria Ag throughout infection with PbA.

We have also analyzed lymphocytes in the brain. The number of CD8$^+$ T cells retained within the brain was increased in mice infected with WT-PbA or OVA-PbA (Fig. 3A). A large variation in the number of CD8$^+$ T cells in the infected mice might reflect their differential stages of cerebral malaria. OT-I cells, however, were increased only in mice infected with OVA-PbA. The number of CD4$^+$ T cells was not significantly different between these groups of mice. We also examine the CFSE profile and surface phenotype of OT-I cells that were sequestered in the brain (Fig. 3B). OT-I cells in the brain of OVA-PbA-infected mice uniformly showed low levels of CFSE and CD44$^{hi}$CD62L$^{lo}$ phenotype, indicating that only highly activated OT-I cells were retained within the brain of mice infected with OVA-PbA.

Activation of malaria-specific CD8$^+$ T cells is TAP dependent
To determine whether the Ag presentation of OVA epitope to OT-I CD8$^+$ T cells utilize the conventional class I Ag presentation pathway, we examined the requirement for the TAP molecule in proliferation of OT-I CD8$^+$ T cells in vivo in TAP null mice (TAP-KO; Fig. 4, A and C). In this experimental system, we monitored the T cell response in vivo within 3 days after transfer, given that the number of OT-I CD8$^+$ T cells was severely reduced 5 days after transfer, perhaps due to their rejection in TAP-KO host mice (data not shown). Thus, we infected B6 or TAP-KO mice with WT-PbA or OVA-PbA, adoptively transferred CFSE-labeled CD8$^+$ T cells from CD45.1$^+$ OT-I mice 5 days later, and monitored proliferation of OT-I T cells by the sequential loss of CFSE intensity 3 days later (parasitemia 2.3–5.4%). Division of OT-I cells was not detectable in uninfected or WT-PbA-infected hosts during these 3 days. In B6 mice infected with OVA-PbA, however, OT-I cells divided several times, indicating that they were activated in vivo in an Ag-specific manner. In TAP-KO hosts, no proliferative responses were observed, indicating that Ag presentation of the OVA epitope expressed in OVA-PbA in association with the MHC class I molecule, was dependent on the TAP molecule. However, OT-I CD8$^+$ T cells showed increased expression of CD69 and reduced expression of CD62L in the TAP-KO host mice infected with WT-PbA or OVA-PbA, suggesting that these phenotypical changes of OT-I CD8$^+$ T
cells could be induced without TCR occupancy during PbA infection (Fig. 4, B and C). We also evaluated their expression of granzyme B by intracellular staining in this model. The expression of granzyme B was undetectable in OT-I CD8+ T cells in uninfected mice but was detected in OT-I CD8+ T cells in WT-PbA or OVA-PbA-infected mice both before and after culture (Fig. 4, D and E). The level of granzyme B expression in OT-I CD8+ T cells ex vivo was higher in OVA-PbA-infected mice than in WT-PbA-infected mice. OT-I CD8+ T cells transferred into TAP-KO mice also showed up-regulation of granzyme B expression, suggesting that it could be induced without TCR occupancy during malaria infection.

Involvement of NK cells in nonspecific activation of CD8+ T cells
It has been reported that NK cells are activated during malaria infection and play pivotal roles in the induction and recruitment of specific CD8+ T cells (31–33). To examine whether CD4+ T
cells, other CD8\(^+\) T cells, or NK cells are involved in the activation of OT-I CD8\(^+\) T cells, we infected RAG2-KO OT-I mice, which lack an adaptive immune system except for monoclonal OVA-specific OT-I CD8\(^+\) T cells, with PbA. OT-I CD8\(^+\) T cells showed clear up-regulation of the CD69 marker in mice infected with WT-PbA or OVA-PbA, indicating that OT-I CD8\(^+\) T cells did not require CD4\(^+\) T cells or other CD8\(^+\) T cells for their activation (Fig. 5A). The expression of granzyme B was also detected in OT-I CD8\(^+\) T cells in RAG2-KO OT-I mice that were infected with WT-PbA or OVA-PbA, indicating that the help of CD4\(^+\) T cells or other CD8\(^+\) T cells was not required for the induction of granzyme B (Fig. 5B). When NK cells were depleted by treatment with anti-NK1.1 mAb in vivo, up-regulation of CD69 and the expression of granzyme B were severely impaired in CD8\(^+\) T cells from WT-PbA-infected mice (Fig. 5). OT-I CD8\(^+\) T cells, however, were activated in NK1.1-treated OVA-PbA-infected mice at levels indistinguishable from the control group, indicating that NK cells are not required for Ag-specific activation of CD8\(^+\) T cells during blood stage infection with PbA. It was reported that NK markers are expressed in T cells of virus-infected mice (34). In RAG2-KO OT-I mice, ~14 and 21% of CD69\(^+\)CD8\(^+\) T cells became NK1.1\(^+\) during infection with WT-PbA and OVA-PbA, respectively (data not shown). Therefore, the effect of NK1.1 mAb on WT-PbA-infected mice was not simply due to the direct depletion of activated CD8\(^+\) T cells. In addition, the reduction of the activated OT-I CD8\(^+\) T cells was seen in WT-PbA-infected mice and not in OVA-PbA-infected mice. Taken together, these results suggested that NK cells were involved in Ag-nonspecific activation of CD8\(^+\) T cells during PbA infection.

![Figure 5](link-to-figure5)

**FIGURE 5.** Involvement of NK cells in the nonspecific activation of OT-I CD8\(^+\) T cells during infection with PbA. RAG2-KO OT-I mice were inoculated with PBS or anti-NK1.1 mAb on -1, 2, 5, and 7 days after infection with WT-PbA or OVA-PbA. On day 8, spleen cells were stained with PE-anti-CD8 and FITC-labeled activation markers (CD69, CD62L, CD44; solid line; A), or with FITC-anti-CD8 and PE-anti-granzyme B (Gzm B; B). Data represent staining profiles of CD8\(^+\)-gated populations. Levels of parasitemia: WT-PbA, PBS-treated (18.2%), NK-depleted (14.2%). Values are representative data of two similar results.

![Figure 6](link-to-figure6)

**FIGURE 6.** Nonspecific activation of CD8\(^+\) T cells from P14 TCR-transgenic mice. Eight days after infection with WT-PbA, spleen cells from P14 mice were stained with anti-CD8 and FITC-labeled activation markers (CD69, CD62L, CD44) or PE-anti-granzyme B (Gzm B). Data represent staining profiles of CD69, CD62L, and CD44 (solid line) and granzyme B of CD8\(^+\)-gated populations. The level of parasitemia was 15.7%.

![Figure 7](link-to-figure7)

**FIGURE 7.** CTL activity in vitro of CD8\(^+\) T cells during PbA-infection. A, B6 mice were uninfected or infected with WT-PbA or OVA-PbA. Five days later, mice were inoculated with CD8\(^+\) T cells (1.1 \times 10^7) from CD45.1 OT-I mice. Three days later, CD8\(^+\) T cells were purified by negative selection (>83%) and were subjected to 51Cr release assay using OVA-pulsed (1 \mu g/ml) and unpulsed (0) EL4 targets for 4 h. Portions of OT-I cells in CD8\(^+\) T cells: uninfected, 8.0%; WT-PbA, 11.0%; OVA-PbA, 31.1%. Levels of parasitemia: WT-PbA, 3.4%; OVA-PbA, 4.0%. B, RAG2-KO OT-I mice were infected with WT-PbA or OVA-PbA. Eight days later, purified CD8\(^+\) T cells were subjected to 51Cr release assay as in A. The number of OT-I cells in CD8\(^+\) T cells was determined based on the percent of OVA-pulsed EL4 tetramer-positive cells. Levels of parasitemia: WT-PbA, 5.9%; OVA-PbA, 5.6%.
To determine whether OT-I CD8\(^+\) T cells that are activated during Plasmodium infection are able to kill targets, we performed CTL assays in vitro. B6 mice were transferred with OT-I CD8\(^+\) T cells and were infected with WT-PbA or OVA-PbA. CD8\(^+\) T cells were enriched from these mice and were subjected to \(^{51}\)Cr release assay (Fig. 7A). OT-I CD8\(^+\) T cells in OVA-PbA-infected B6 mice showed specific CTL activity against OVA-pulsed targets. OT-I CD8\(^+\) T cells from WT-PbA-infected mice showed weak but significant OVA-specific killing activity. We also examined CTL activity of CD8\(^+\) T cells in RAG2-KO OT-I mice. These cells showed OVA-specific CTL activity after infection with WT-PbA or OVA-PbA, indicating that CTL can be induced without help of CD4\(^+\) or other CD8\(^+\) T cells (Fig. 7B). The CTL activity of OT-I CD8\(^+\) T cells from OVA-PbA-infected mice was much higher than those from WT-PbA-infected mice, consistent with their higher expression of granzyme B (Fig. 5B).

We also evaluated in vivo killing activity of CD8\(^+\) T cells during malaria infection. B6 mice were transferred or not transferred with OT-I CD8\(^+\) T cells and were infected with WT-PbA or OVA-PbA. Seven days after the infection, these mice received splenocytes that were differentially labeled with CFSE and were left unpulsed (CFSE\(^{low}\)) or pulsed (CFSE\(^{high}\)) with OVApep. The spleen cells were analyzed 4 h later (Fig. 8). OVApep-coated target cells were specifically and almost completely cleared within 4 h in OT-I CD8\(^+\) T cell-transferred OVA-PbA-infected mice. Peptide-pulsed targets were also significantly reduced in OT-I CD8\(^+\) T cell-transferred WT-PbA-infected mice, although the levels of reduction were much less than those in OVA-PbA-infected mice. These effects were not seen in OT-I CD8\(^+\) T cell-transferred unpulsed OVApep-infected mice or in the infected mice without OT-I CD8\(^+\) T cell transfer.

Pathogenesis of CD8\(^+\) T cells activated by malaria infection

To determine the role of CD8\(^+\) T cells activated during malaria infection, B6, RAG2-KO, and RAG2-KO OT-I mice were infected with WT- or OVA-PbA (Fig. 9). B6 mice died 8–12 days after infection with WT- or OVA-PbA with clinical signs of cerebral malaria. Although the incidence of the cerebral malaria in B6 mice was relatively low (60–80%), it was within the range reported by Amani et al. (35). RAG2-KO mice did not develop cerebral malaria and survived >30 days after infection with WT- or OVA-PbA, consistent with previous studies indicating the requirement for CD8\(^+\) T cells in the development of cerebral malaria (7–11).
RAG2-KO OT-I mice were resistant to WT-PbA infection, similar to RAG2-KO mice, and survived >30 days after infection, suggesting that nonspecific activation of CD8⁺ T cells is not by itself harmful to the host. However, RAG2-KO OT-I mice showed levels of parasitemia higher than RAG-2 KO mice and three of five mice died 13–29 days after infection with OVA-PbA. Although statistical analysis of these data showed that the difference in survival time was not significant between RAG2-KO OT-I and RAG2-KO mice in this particular experiment, we think that it is likely due to the small number of the mice used in this experiment. We observed similar data in another set of experiments; six of seven RAG-2 KO OT-I mice died 15–29 days after infection with OVA-PbA, whereas none of RAG-2 KO mice died within 30 days after infection. Taken together, these data suggest that the activation of malaria-specific CD8⁺ T cells, in the absence of a diverse adaptive immune system, could lead to the development of lethal pathogenesis during infection with blood stage PbA.

Discussion

This study indicated using a model malaria Ag, OVA, that malaria Ag can be presented to specific CD8⁺ T cells by APCs in TAP-dependent cross-presentation during infection with PbA. The requirement of TAP for this pathway suggests that cross-presentation of malaria Ags involves the phagosome-to-cytosol pathway, in which Ags are exported to the cytosol after engulfment and are transported into the ER via TAP molecules, as reported for some other microorganisms such as M. tuberculosis and T. gondii (12, 13, 18, 19). Although infection with the malaria parasite might modulate the function of DCs or inhibit cross-presentation (30, 36–38), our study clearly indicates that APCs are able to cross-present malaria Ags that they have engulfed and activate specific CD8⁺ T cells during the erythrocyte stage of malaria infection. Although we used a model Ag, OVA, it is likely that the endogenous malaria Ags are presented in a similar manner. The identification of natural CTL epitopes expressed in the erythrocyte stage of malaria parasites would aid our understanding of the role of CD8⁺ T cells against the malaria blood stage.

In addition to Ag-specific response of CD8⁺ T cells, we have found that Ag-nonspecific CD8⁺ T cells could proliferate, show activation phenotype, express granzyme B, and gain CTL function when the host mice were infected with PbA, albeit at a lower level. A couple of possibilities might account for this nonspecific activation of OT-I CD8⁺ T cells. First, OT-I CD8⁺ T cells might directly recognize the PbA epitope by cross-reactivity of their TCR. We think that this possibility is unlikely, because OT-I CD8⁺ T cells were activated not only in B6, but also in TAP-KO hosts, which are defective in the phagosome-to-cytosol pathway of Ag presentation, suggesting that the activation of OT-I CD8⁺ T cells in vivo by WT-PbA did not require TCR engagement. In addition, a similar activation-phenotype was observed in CD8⁺ T cells of P14 TCR-transgenic mice as well as other RAG2-KO TCR-transgenic mice during infection with malaria parasites (Fig. 6 and unpublished observations). Second, host CD8⁺ T cells might be activated by parasite products via interaction with their receptors other than TCR. Naive and activated CD8⁺ T cells express a variety of pathogen-recognizing receptors including TLRs (39). Engagement of these receptors with ligands derived from parasites might modulate T cell function without TCR signaling. In particular, it is known that TLR2 is expressed on activated T cells and exhibits costimulatory function for TCR-stimulated T cells or can directly induce Th1 effector function (40, 41). Malaria parasites express GPI anchors that are recognized by TLR2 (42), thus possibly directly modulating the function of host T cells. However, the activation of naive CD8⁺ T cells by TLR stimulation has not been reported. A third possibility is that CD8⁺ T cells are activated by cytokine(s) produced by the innate immune system in response to PbA infection (43–45). Our study suggested that NK cells are involved in nonspecific activation of CD8⁺ T cells. NK cells produce cytokines such as IFN-γ and TNF-α during malaria infection (31). Naive T cells can be activated by cytokines without TCR engagement, which has been termed the innate T cell activation pathway (46). Taken together, it is likely that cytokines produced by NK cells, in combination with products of malaria parasites, participate in Ag-nonspecific activation of CD8⁺ T cells during infection with PbA.

We demonstrated that two types of CD8⁺ T cells are activated during malaria infection: those specific for malaria Ag and activated by TAP-dependent Ag presentation; and those activated nonspecifically. In both types of activation, CD8⁺ T cells express the activation phenotype and granzyme B and can develop into functional CTL, although the levels of the nonspecific activation are much lower than the specific activation. Our study suggested that CD8⁺ T cells that are activated in an Ag-specific manner are involved in the pathogenesis of severe malaria. Highly activated OT-I CD8⁺ T cells preferentially sequestered in the brain of B6 mice that were transferred with OT-I cells and infected with OVA-PbA (Fig. 3). In this experiment, however, it was unclear whether these cells were involved in the pathogenesis of cerebral malaria, since host B6 CD8⁺ T cells were sufficient to cause cerebral malaria. In contrast, RAG2-KO OT-I mice that were infected with OVA-PbA showed early death when compared with RAG2-KO mice, suggesting that activation of OT-I CD8⁺ T cells was pathogenic to the host, likely due to bystander mechanisms (Fig. 9). OVA-PbA-infected RAG2-KO OT-I mice showed more severe parasitemia and died later than B6 mice, suggesting that the death of RAG2-KO mice was not caused by cerebral malaria but may have been caused by other pathological processes associated with the infection. Taken together, these results suggest that the activation of malaria-specific CD8⁺ T cells can be pathogenic to the host, but the development of cerebral malaria may require additional factors as has been discussed (10, 11). On the other hand, RAG2-KO OT-I mice showed a clinical course indistinguishable from RAG2-KO mice when infected with WT-PbA, suggesting that CD8⁺ T cells that are activated in an Ag-nonspecific manner are generally not pathogenic to the host. Nonspecific activation of CD8⁺ T cells, however, does not require TCR engagement and thus might include a pool of peripheral CD8⁺ T cells that recognize various MHC class I-bound epitopes including self-Ag. Therefore, it remains possible that activation and CTL development of the self-reactive pool of peripheral CD8⁺ T cells could lead to the destruction of tissue and might be involved in the pathogenesis of malaria. Further studies on the molecular mechanisms underlying the malaria-specific and nonspecific activation of CD8⁺ T cells are important for expanding our understanding of protection against Plasmodium infection and of the pathogenesis of severe malaria.

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Disclosures

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References


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