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Coinfection with Nonlethal Murine Malaria Parasites Suppresses Pathogenesis Caused by *Plasmodium berghei* NK65

Mamoru Niikura,†‡ Shigeru Kamiya, † Kiyoshi Kita, ‡ and Fumie Kobayashi†‡

Mixed infection with different *Plasmodium* species is often observed in endemic areas, and the infection with benign malaria parasites such as *Plasmodium vivax* or *P. malariae* has been considered to reduce the risk of developing severe pathogenesis caused by *P. falciparum*. However, it is still unknown how disease severity is reduced in hosts during coinfection. In the present study, we investigated the influence of coinfection with nonlethal parasites, *P. berghei* XAT (*Pb* XAT) or *P. yoelii* 17X (*Py* 17X), on the outcome of *P. berghei* NK65 (*Pb* NK65) lethal infection, which causes high levels of parasitemia and severe pathogenesis in mice. We found that the simultaneous infection with nonlethal *Pb* XAT or *Py* 17X suppressed high levels of parasitemia, liver injury, and body weight loss caused by *Pb* NK65 infection, induced high levels of reticulocytopenia, and subsequently prolonged survival of mice. In coinfected mice, the immune response, including the expansion of B220intCD11c+ cells and CD4+ T cells and expression of IL-10 mRNA, was comparable to that in nonlethal infection. Moreover, the suppression of liver injury and body weight loss by coinfection was reduced in IL-10−/− mice, suggesting that IL-10 plays a role for a reduction of severity by coinfection with nonlethal malaria parasites. *The Journal of Immunology*, 2008, 180: 6877–6884.

**Materials and Methods**

**Mice**

Female C57BL/6 (B6) mice were purchased from CLEA Japan and used at 5–6 wk of age. IL-10−/− mice on B6 background were purchased from The Jackson Laboratory. We used 20- to 24-wk-old female IL-10−/− mice (experiment 1), 5- to 6-wk-old male or female IL-10−/− mice (experiment 2), and age-matched female B6 mice in these studies. The genotype of female IL-10−/− mice used in experiments was verified by PCR. The experiments were approved by the Experimental Animal Ethics Committee at Kyorin University, and all experimental animals were kept on the specific pathogen-free unit at the animal facility with sterile bedding, food, and water.

**Parasites and infections**

Malaria parasites were stored as frozen stocks in liquid nitrogen. *Pb* NK65 is a high-virulence strain and was originally obtained from Dr. M. Yoei (New York University Medical Center, New York, NY). *Pb* XAT is a low-virulence derivative from *Pb* NK65 (15). A nonlethal isolate of *Py* 17X was originally obtained from Dr. J. Finnerty (National Institutes of Health, Bethesda, MD) and cloned by limiting dilution. Parasitized RBCs (pRBCs) of *Pb* NK65, *Pb* XAT, or *Py* 17X were generated in donor mice inoculated i.p. with each frozen stock of parasites. The donor mice were monitored for parasitemia daily and bled for experimental infection in ascending periods of parasitemia. Experimental mice were infected i.v. with 1 × 10⁴ pRBCs of a given parasite species or strain. Therefore, when mice were coinfected with two species/strains of parasites, a total of 2 × 10⁴ pRBCs (1 × 10⁴ of each parasite species/strain) were inoculated.

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2. Abbreviations used in this paper: *Pb*, Plasmodium berghei; *Py*, Plasmodium yoelii; pRBC, parasitized RBC; AST, aspartic aminotransferase; ALT, alanine aminotransferase.

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Parasitemia
Parasitized RBCs were observed by microscopic examination of methanol-fixed tail blood smears stained for 45 min with 1% Giemsa diluted in phosphate buffer (pH 7.2). The number of pRBCs in 250 RBCs was enumerated when parasitemia exceeded 10%, whereas 1 × 10^4 RBCs were examined when mice showed lower parasitemia. The percentage of parasitemia was calculated as follows: [(No. of pRBCs)/(Total no. of RBCs counted)] × 100.

Measurement of body weights, hematocrits, and circulating reticulocytes
Body weights were measured by balance for animals (KN-661; Natsume), and body weight loss was expressed as a percentage of the day 0 value. For hematocrit measurement, tail blood (50 μl) was collected into a heparinized capillary tube and centrifuged at 13,000 × g for 5 min with a microhematocrit centrifuge (HC-12A; Tomy). The hematocrit value was expressed as a percentage of the total blood volume. Reticulocytes in 250 RBCs were counted when reticulocytemia exceeded 20%, whereas 1 × 10^4 RBCs were examined when mice showed lower reticulocytemia. The percentage of reticulocytemia was calculated as follows: [(No. of reticulocytes)/(Total no. of RBCs counted)] × 100.

Histological examination and measurement of parameters of liver injury
Livers were obtained from infected mice on day 9 postinfection and fixed with 1% paraformaldehyde. Two-color flow cytometry was performed and analyzed with a FACSCalibur (BD Biosciences) using a flowJo software (version 7.1.3, for Windows).

Detection of cytokine mRNA in spleens
Spleens were removed from infected mice on day 9 postinfection and total RNA was isolated by bogen (Nippon Gene) according to the manufacturer’s protocol. The splenic RNA was reverse-transcribed by murine leukemia virus reverse transcriptase (Applied Biosystems) using random hexamer primers, and reverse transcriptase reaction was performed at 70°C for 10 min, at 25°C for 10 min, and at 42°C for 30 min. The reaction was terminated by heating at 99°C for 5 min, and the cDNA products were stored at −20°C until use. The 50 μl PCR mixture contained 1 × TaKaRa Ex Taq buffer, 2.5 mM dNTP, 1 μl cDNA products, 5 U/μl TaKaRa Ex Taq DNA polymerase, and 0.25 μM of PCR primers. The primers used for PCR amplification were as follows: IL-10, 5'-GTG AAG ACT TTC TTT GCG GTG CA. Thirty cycles of PCR were performed on a thermal cycler (iCycler; Bio-Rad). Each cycle consisted of 30 s of denaturation at 94°C, 30 s of annealing at 60°C, and 1 min of elongation at 72°C. The PCR products were analyzed on a 2% agarose gel stained with ethidium bromide.

Cytokine assay
An ELISA for the detection of IFN-γ or IL-10 in plasma was conducted as described previously (16). A rat anti-mouse IFN-γ (clone R4–6A2; eBioscience) and a rat anti-mouse IL-10 (clone JES5–16E3; eBioscience) were used as the capture Abs, and a biotin-conjugated rat anti-mouse IFN-γ (clone XMG1.2; eBioscience) and IL-10 (clone JES5-2A5; eBioscience) were used as the detecting Abs. The concentration of cytokines in plasma was calculated from standard curves prepared with known quantities of murine recombinant IFN-γ (Genzyme) and murine recombinant IL-10 (Pierce).

Statistical analysis
For time-series comparisons, Student’s t test and one- and two-way ANOVAs with Fisher’s PLSD post hoc test were performed using Statcel program (OMS). Survival curves were compared using a log-rank test, p < 0.05 was set as statistical significance of differences.

Results
Infection with Pb XAT but not Pb 17X induces protective immunity to Pb NK65
It has been shown that mice infected with Pb NK65 develop severe parasitemia and die within 2 wk, although mice infected with Pb XAT or Py 17X cure spontaneously around 3 wk of infection (15, 17). To examine whether primary infection with each of the two nonlethal parasites can induce protective immunity against Pb NK65 lethal infection, groups of C57BL/6 (B6) mice were infected with Pb XAT or Py 17X then challenged with Pb NK65 on day 30 after primary infection. As expected, mice cured from Pb XAT infection (Pb XAT-immunized mice) showed extremely low levels of parasitemia after secondary infection with Pb NK65 (Fig. 1A). On the contrary, mice cured from Py 17X infection (Py 17X-immunized mice) showed high levels of parasitemia, with some delay in onset of parasitemia, and eventually died after Pb NK65 infection (Fig. 1B). These results suggest that protective immunity to Pb NK65 is induced by immunizing mice with Pb XAT but not with heterologous Py 17X.

Table I. Total spleen cell number in uninfected and infected mice (×10^6)a

<table>
<thead>
<tr>
<th>Days Postinfection</th>
<th>Uninfected</th>
<th>Pb NK65</th>
<th>Pb XAT</th>
<th>Pb NK65/Pb XAT</th>
<th>Py 17X</th>
<th>Pb NK65/Py 17X</th>
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<tr>
<td>0</td>
<td>0.35 ± 0.17</td>
<td>1.05 ± 0.16</td>
<td>0.74 ± 0.08</td>
<td>0.74 ± 0.10</td>
<td>0.99 ± 0.18</td>
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*a Mice were infected with malarial parasites as described in the legend to Fig. 2. Spleens were obtained from uninfected and infected mice on days 6, 9, and 15 after infection. Results are expressed as means ± SD of three mice. Experiments were performed three times with similar results.
infected mice during early infection (Fig. 2A) and survived significantly longer than did Pb NK65 singly infected mice (Fig. 2B) \((p = 0.0013)\). Moreover, the body weight loss of the coinfected mice was prevented early in infection (Fig. 2C) \((p < 0.0005)\) compared with Pb NK65-infected mice on days 9–10. Next, we examined the influence of coinfection with nonlethal Py 17X on the outcome of Pb NK65 infection. Although Py 17X immunization did not affect the outcome of Pb NK65 infection greatly (Fig. 1B), simultaneous infection with Py 17X (Pb NK65/Py 17X) suppressed severe parasitemia, mortality \((p = 0.0005)\), and the body weight loss \((p < 0.0005)\) on days 6–10 observed in Pb NK65 singly infected mice (Fig. 2, D–F).

**Coinfection with nonlethal malaria parasites induces reticulocytemia**

To examine whether the existence of nonlethal malaria parasites affects the severe anemia caused by Pb NK65 infection, we determined the hematocrit in mice during Pb NK65 single infection and coinfection with Pb XAT or Py 17X. Coinfection with Pb XAT caused acute anemia as severe as did Pb NK65 single infection on day 9 postinfection, and the levels of hematocrit were also low on day 15 (Fig. 3A). Mice infected with Pb XAT did not cause acute severe anemia. In contrast, mice coinfeiced with Pb NK65/Py 17X did not cause as severe anemia as for Pb NK65-infected mice on day 9, and their reducing pattern of hematocrit was similar to that in Py 17X singly infected mice (Fig. 3C).

Next, we determined the reticulocytemia in infected mice. Pb NK65 singly infected mice did not show any reticulocytemia during infection (Fig. 3B). Mice coinfeiced with Pb NK65/Pb XAT showed the same levels of reticulocytemia as did Pb XAT singly infected mice on day 9 postinfection. However, coinfeiced mice showed much higher reticulocytemia than that in Pb XAT singly infected mice from day 11 postinfection (Fig. 3B). As shown in Fig. 3D, reticulocytemia in mice coinfeiced with Pb NK65/Py 17X increased moderately, and their kinetics were similar to those in Py 17X singly infected. These results indicate that the severe anemia caused by Pb NK65 infection is suppressed by coinfection with Py 17X but not with Pb XAT. It is suggested that high levels of reticulocytemia observed during Pb NK65/Pb XAT infection may

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**FIGURE 1.** Immunization with Pb XAT but not Py 17X induces protective immunity to PHL NK65. C57BL/6 mice were infected with \(1 \times 10^4\) pRBCs of Pb XAT (A) or Py 17X (B) (day 0, open arrows). On day 30 after primary infection (filled arrows), both groups of mice were challenged with \(1 \times 10^4\) pRBCs of Pb NK65. A. Course of parasitemia in immunized mice with Pb XAT (○). B. Course of parasitemia in immunized mice with Py 17X (●). Course of parasitemia of unimmunized mice infected with Pb NK65 is inserted to figures (shaded triangles). Results are expressed as mean percentage parasitemia ± SD of three mice. Experiments were performed three times with similar results.

**The pathogenesis during Pb NK65 infection is reduced by simultaneous infection with nonlethal malaria parasites**

To investigate whether the existence of nonlethal malaria parasite affects the outcome of Pb NK65 infection, B6 mice were infected with Pb NK65 and nonlethal parasites simultaneously. When mice were coinfeiced with Pb NK65 and Pb XAT (Pb NK65/Pb XAT), they showed lower levels of parasitemia than did Pb NK65 singly

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**FIGURE 2.** Coinfection of nonlethal Pb XAT or Py 17X suppresses the acute severe parasitemia and body weight loss caused by Pb NK65 infection in mice and prolonged their survival. C57BL/6 mice were inoculated with \(1 \times 10^4\) pRBCs of Pb NK65, Pb XAT, or Py 17X. When mice were coinfeiced with two species/strains of parasites, a total of \(2 \times 10^4\) pRBCs were inoculated (Pb NK65/Pb XAT or Pb NK65/Py 17X). Results of coinfection are shown for Pb NK65/Pb XAT (A–C) or Pb NK65/Py 17X (D and E). A and D, Course of parasitemia. Asterisks indicate statistically significant differences \((*, p < 0.001)\) as compared with Pb NK65-infected mice). B and E, Survival rates. Differences between Pb NK65 singly infected mice and coinfeiced mice are statistically significant \((p < 0.001)\). C and F, Body weights. Asterisks indicate statistically significant differences \((*, p < 0.001)\) as compared with Pb NK65-infected mice). Results are expressed as means ± SD of five mice. Experiments were performed three times with similar results.
be induced by severe anemia. The different outcome of the suppression of anemia and reticulocytemia between \( \text{Pb}^{\text{NK65/\\text{Pb}^XAX}} \) and \( \text{Pb}^{\text{NK65/\\text{Py}^{17X}}} \)-infected mice might be attributed to the difference in parasitemia during early infection.

Low levels of liver injury in mice coinfected with nonlethal malaria parasites

To investigate whether the existence of nonlethal malaria parasites affects the liver injury caused by \( \text{Pb}^{\text{NK65}} \) infection, we performed histological examination of livers from mice during \( \text{Pb}^{\text{NK65}} \) single infection and coinfection with \( \text{Pb}^{\text{XAT}} \) or \( \text{Py}^{17X} \). As shown in Fig. 4, focal necrosis of the liver cells (Fig. 4, B and F, arrowheads) and dense infiltration of inflammatory cells such as mononuclear cells around the portal tracts (Fig. 4F, arrows) were observed in \( \text{Pb}^{\text{NK65}} \)-infected mice. Mice coinfected with \( \text{Pb}^{\text{NK65/\\text{Pb}^{XAT}}} \) or \( \text{Pb}^{\text{NK65/\\text{Py}^{17X}}} \) also showed dense infiltration of inflammatory cells (Fig. 4, G and H, arrows), but focal necroses were not observed in the liver (Fig. 4, C and D).

We determined the levels of AST and ALT, which are parameters of liver injury, in the plasma. \( \text{Pb}^{\text{NK65}} \)-infected mice, in which the focal necroses of liver cells were observed, showed the significantly high concentration of AST and ALT compared with uninfected control mice (Fig. 4, I and J). The levels of AST and ALT in coinfected mice were quite low and almost the same as those in \( \text{Pb}^{\text{XAT}} \) or \( \text{Py}^{17X} \) singly infected mice.

Coinfection with nonlethal parasites accelerates \( \text{B220}^{\text{int/CD11c^{+}}} \) cell expansion in spleen and peripheral blood

To examine the expansion of the \( \text{CD11c}^{+} \) cell populations during malaria, additional experiments were performed using peripheral blood and spleen obtained from infected mice by flow cytometry in each time point after infection. It was notable that the \( \text{B220}^{\text{int/CD11c^{+}}} \) cell population significantly increased in peripheral blood from \( \text{Pb}^{\text{NK65/\\text{Pb}^{XAT}}} \) or \( \text{Pb}^{\text{NK65/\\text{Py}^{17X}}} \)-coinfected mice on day 6 postinfection (Fig. 5, A, upper panels). Their expansion was comparable to that observed in \( \text{Pb}^{\text{XAT}} \) or \( \text{Py}^{17X} \) singly infected mice.
single infection, respectively (Fig. 5C). The B220<sup>+</sup>CD11c<sup>+</sup> cell population in those four groups of mice decreased on day 9 postinfection (Fig. 5A, lower panels). Although B220<sup>+</sup>CD11c<sup>+</sup> cells in Pb NK65-infected mice also expanded on day 6 postinfection, they were much less than those in coinfected or nonlethal parasite-infected mice. The cell population in Pb NK65-infected mice further expanded on day 9 postinfection, when no other groups of mice showed the expansion (Fig. 5A). The B220<sup>+</sup>CD11c<sup>+</sup> cell population of spleen showed a similar pattern to that of peripheral blood (Fig. 5B), but the proportion of the cells in Pb NK65/Pb XAT-infected mice was lower than that in Pb XAT-infected mice on day 6 postinfection (Fig. 5D). These results suggested that coinfection with nonlethal parasites accelerated much more B220<sup>+</sup>CD11c<sup>+</sup> cell expansion than did Pb NK65 single infection during the early phase of infection.

**Coinfection with nonlethal parasites induces CD4<sup>+</sup> T cell expansion in spleen**

We analyzed the kinetics of CD4<sup>+</sup> T cell expansion in spleen during single and mixed infection (Fig. 6). Significant expansion of splenic CD4<sup>+</sup> T cells in Pb XAT- or Py 17X-infected mice was observed from day 9 postinfection. In contrast, Pb NK65-infected mice did not show the increased levels of CD4<sup>+</sup> T cells even on day 9 postinfection. Mice coinfected with Pb NK65/Pb XAT or Pb NK65/Py 17X had almost the same number of splenic CD4<sup>+</sup> T cells as did Pb XAT- or Py 17X-infected mice, respectively.

**Enhanced levels of IL-10 mRNA during coinfection and nonlethal infection**

IFN-γ and IL-10 have been shown to be associated with protection and exacerbation during *P. berghei* and *P. yoelii* malaria (17, 18).

**FIGURE 5.** Coinfection with nonlethal parasites accelerates B220<sup>+</sup>CD11c<sup>+</sup> cell expansion in spleen and peripheral blood. Peripheral blood and spleen were obtained from infected mice as described in the legend to Fig. 2 on days 6 and 9 after infection and from uninfected mice. Analyses of CD11c<sup>+</sup> cell population in peripheral blood (A and C) and spleen (B and D) from infected mice were performed by flow cytometry. Expression of B220 and CD11c was analyzed in the gate of CD3<sup>-</sup>. A and B, Contour plots of B220<sup>+</sup>CD11c<sup>+</sup> cell population (day 6, upper panels; day 9, lower panels), p.i., Post infection. Experiments were performed three times with similar results and the representative results are shown. C and D, The proportion of B220<sup>+</sup>CD11c<sup>+</sup> cells in CD3<sup>-</sup> cells is shown (on day 6 postinfection). Asterisks indicate a statistically significant difference (*, *p* < 0.005; **, *p* < 0.001 as compared with Pb NK65-infected mice). Results are expressed as means ± SD of three mice.

**FIGURE 6.** Coinfection with nonlethal parasites induces CD4<sup>+</sup> T cell expansion in spleen. Spleens were obtained from infected mice as described in the legend to Fig. 2 on days 6, 9, and 15 after infection and from uninfected mice. Analyses of CD3<sup>+</sup>CD4<sup>+</sup> cells in spleen were performed by flow cytometry and total numbers of CD4<sup>+</sup> T cells in spleen were calculated. Asterisks indicate a statistically significant difference (*, *p* < 0.05; **, *p* < 0.005 as compared with uninfected control mice). Results are expressed as means ± SD of three mice. Experiments were performed three times with similar results.
To examine whether these cytokines are associated with the suppression of *Pb* NK65-caused pathogenesis by coinfection with nonlethal malaria parasites, we determined the levels of cytokines in plasma and cytokine mRNA in spleens from singly infected or coinfected mice as described in the legend to Fig. 2 on day 9 and subjected to RT-PCR using cytokine-specific primers. The samples without RNA template were used as negative control. Note that *IL-10* mRNA was not detected in spleen from uninfected mice. These results led us to hypothesize that the enhanced levels of *IL-10* may be involved in suppression of pathogenesis during coinfection.

**IL-10-deficient mice fail to receive benefits by coinfection with nonlethal malaria parasites**

To examine whether *IL-10* is associated with the suppression of the pathogenesis caused by coinfection, we determined the parasite-sitemia, mortality, and the body weight of *Pb* NK65-infected *IL-10*−/− mice coinfected with *Pb* XAT or *Pb* 17X. *Pb* NK65/Pb XAT-coinfected wild-type mice survived by day 21 (Fig. 8D), confirming the data obtained in Fig. 2B. In contrast, *IL-10*−/− mice coinfected with *Pb* NK65/Pb XAT began to die from day 10, and all mice died by day 21 postinfection (Fig. 8D) (*p* = 0.034). Moreover, their body weights were significantly lower than coinfected wild-type mice (Fig. 8E) (*p* < 0.001 on days 9, 11, and 13), although their parasitemia did not increase from day 11 (Fig. 8F). Similarly, *Pb* NK65/Pb 17X-coinfected *IL-10*−/− mice began to die earlier than did wild-type mice (Fig. 8G), and their body weights were also lower than those of wild-type mice (Fig. 8H) (*p* < 0.001 on days 9, 13, and 18). During the period when coinfected *IL-10*−/− mice began to die, they developed liver injury (Fig. 8, M and O), which was not observed in coinfected wild-type mice (Fig. 8, L and N). In contrast, the parasitemia, mortality, the body weight, and development of liver injury of *Pb* NK65 singly infected *IL-10*−/− mice were not different from those of wild-type mice (Figs. 8, A–C, J, and K). Altogether, these results suggest that *IL-10* may be involved in the suppressive effect of coinfection with nonlethal malaria parasites on the outcome of lethal *Pb* NK65 infection.

**Discussion**

In the present study, we investigated the influence of simultaneous infection with nonlethal murine malaria parasites, *Pb* XAT or *Pb* 17X, on the outcome of the lethal *Pb* NK65 infection. *Pb* NK65 infection caused acute high parasitemia and pathogenesis, including body weight loss, severe anemia, and liver injury in mice. We found herein that the coinfection with nonlethal *Pb* XAT or *Pb* 17X reduced such pathogenesis caused by *Pb* NK65 infection and prolonged survival of mice (Figs. 2–4). Because low levels of parasitemia and body weight loss in coinfected mice were observed from day 6 to 7 (Fig. 2), we postulated that T/B cell-mediated immunity would be involved in the suppressive effects of simultaneous infection with nonlethal parasites on lethal *Pb* NK65 infection and examined the response of dendritic cells and CD4+ T cells.

The large expansion of B220<sup>hi</sup>CD11c<sup>+</sup> cells was observed in spleen and peripheral blood from coinfected mice on day 6, which was comparable to that from nonlethal parasite-infected mice (Fig. 5). These results suggest that expansion of B220<sup>hi</sup>CD11c<sup>+</sup> cells in coinfected mice may be accelerated by nonlethal parasite relative to lethal parasitic infection. It has been reported that CD11c<sup>+</sup> dendritic cells are one of the professional APCs. As the murine plasmacytoid dendritic cell subset has been shown to coexpress CD11c and B220 (19–20), one would speculate that B220<sup>hi</sup>CD11c<sup>+</sup> cells expanded during nonlethal infection or coinfection might be one of the murine plasmacytoid dendritic cell subpopulations. Further characterization of the B220<sup>hi</sup>CD11c<sup>+</sup> cells, however, is needed for identification of these cells. In *Pb* NK65-infected mice, the peak expansion of B220<sup>hi</sup>CD11c<sup>+</sup> cells was observed on day 9, when these cells...
began to decrease in coinfected mice as well as nonlethal Pb XAT- or Py 17X-infected mice. Because Pb NK65 parasites multiply quickly, especially in early phase of infection, earlier expansion of B220<sup>int</sup>CD11c<sup>+</sup> cells may be the key for the suppression of pathogenesis during coinfection.

In contrast, mice coinfected with Pb NK65 and nonlethal Pb XAT or Py 17X showed increased levels of CD4<sup>+</sup> T cells from day 9 that were comparable to nonlethal parasite-infected mice (Fig. 6). Dendritic cells have been shown to activate naive T cells and play a crucial role in the initiation of immune responses (21–23). It is possible that the expansion of splenic CD4<sup>+</sup> T cells might be induced by B220<sup>int</sup>CD11c<sup>+</sup> cells that had been expanded earlier (on day 6), and then the expanded CD4<sup>+</sup> T cells might be involved in suppression of pathogenesis in coinfected mice. CD4<sup>+</sup> T cells have been shown to play both protective and pathological roles during malaria infection (24–25). However, it seems that CD4<sup>+</sup> T cells would play protective roles during coinfection with lethal and nonlethal malaria parasites.

IL-10, which is produced by Th2 cells in CD4<sup>+</sup> T cell categories, inhibits inflammatory cytokines such as IFN-γ, TNF-α (26), and IL-12 (27). In malaria, IL-10 as well as TGF-β has been shown to be critical for host survival during P. berghei ANKA (28, 29) and P. chabaudi AS (30) infection. In the present study, Pb NK65/Pb XAT- or Pb NK65/Py 17X-coinfected mice showed high levels of IL-10 mRNA comparable to those in nonlethal Pb XAT- or Py 17X-infected mice (Fig. 7C), although Pb NK65-infected mice showed only a faint level of IL-10 mRNA. Moreover, high levels of IL-10 in plasma were followed by the IL-10 mRNA expression in coinfected mice on day 15 when IFN-γ production was...
suppressed (Fig. 7). These results suggest that IL-10 may be involved in the suppression of pathogenesis in coinfected mice. As expected, the suppressive effect of coinfection with nonlethal Pb XAT or Py 17X on severe body weight loss, liver injury, and mortality during Pb NK65 infection was reduced in IL-10−/− mice (Fig. 8), suggesting that IL-10 was involved in suppression of exacerbation of infection in simultaneous infection. The excessive inflammation has been shown to be able to account for body weight loss, liver injury, and mortality in mice infected with Pb NK65 (18, 31). Therefore, it is probable that enhancement of IL-10 would have suppressed the excessive inflammation caused by Pb NK65 and subsequently led to suppression of pathogenesis. In contrast, mortality as well as body weight loss in IL-10−/− mice during coinfection were not identical with those in Pb NK65 singly infected IL-10−/− mice, suggesting that other regulatory factors, such as TGF-β (30), may be involved in suppression of pathogenesis.

In the late phase of infection, IL-10−/− mice coinfected with Pb NK65/Pb XAT or Pb NK65/Py 17X had lower levels of parasitemia than that in wild-type mice. These results suggest that although IL-10 plays an important role for suppression of liver injury, it may be also involved in suppression of clearance of malaria parasites and cause death by severe anemia in the late phase of coinfection. It has been shown that during Py 17XL lethal infection, IL-10 is involved in the exacerbation of infection because depletion of IL-10 prolonged survival of hosts and made some mice resolve the infection (17, 32, 33). IL-10 might have dual roles, protective and pathogenic, in mice coinfected with lethal and nonlethal malaria parasites.

Our findings showing the beneficial influence of coinfection with nonlethal Pb XAT or Py 17X to hosts during Pb NK65 infection indicate that suppression of disease severity induced by coinfection occurs in not only cerebral malaria but also pathogenesis such as body weight loss and liver injury. Our data suggest that the beneficial influence of coinfection with nonlethal malaria parasites may not be species-specific because a different species of malaria parasites, Py 17X, also induced protective immunity to Pb NK65 lethal infection by simultaneous infection (Fig. 2). In endemic areas, coinfections have made diagnosis and treatment difficult because host immune responses induced by each of the different Plasmodium spp. are mutually interfered with in a complicated manner. Results obtained from in vivo models of coinfection with murine malaria parasites would contribute to understand the host immune responses during mixed infection with different Plasmodium spp.

Disclosures

The authors have no financial conflicts of interest.

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