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GENETIC CONTROL OF IMMUNITY TO Plasmodium yoelii SPORozoites

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Using a rodent malaria system, we have shown that protective immunity to the preerythrocytic stages of malaria is genetically controlled by MHC and non-MHC genes. Ten congenic strains of mice were immunized with irradiated sporozoites of Plasmodium yoelii. When challenged with viable sporozoites, only two strains had a high proportion of animals that did not develop blood stage infections. Immunity did not correlate with antisporeozoite antibody levels. Two protective mechanisms exist determined by non-H-2 genes, and each mechanism is further controlled by H-2-linked Ir genes. On the BALB background only H-2d mice are protected, and protection is abolished by depleting CD8+ T cells. In contrast, on the B10 background only H-2q mice are strongly protected, and protection is not affected by CD8+ T cell depletion. If similar complex genetic regulation of immunity occurs in the human malarias, it will be a major hurdle for vaccine development.

Malaria infects the mammalian host when mosquitoes inject sporozoites during a blood meal. The parasite develops in the host liver and then enters the blood to infect RBC, the stage in the infection that causes symptoms. Previous inoculation of irradiated sporozoites confers immunity by preventing full development in the liver, which blocks erythrocytic infection and prevents disease (1). Both antibody and T cells can contribute to this immunity. mAb directed against a repeated epitope on the circumsporozoite protein of either Plasmodium berghei (2) or Plasmodium yoelii (Y. Charoenvit, personal communication) protect mice from infection by sporozoites. Recently, experiments in these two rodent malarias have shown that irradiated sporozoites induce CD8+ effector immunization cells that kill the parasite during the preerythrocytic stage (3, 4). A subunit vaccine could potentially stimulate each of these effector mechanisms. However, a successful vaccine must elicit immune responses from most persons regardless of their genetic differences. Hoffman et al. (5) have demonstrated that many congenic strains of mice could be protected against P. berghei sporozoites by immunization with irradiated sporozoites. Therefore immunity to this parasite was not limited by genes of the MHC, called H-2 in the mouse. We now report that immunization of mice with irradiated sporozoites of another rodent malaria, P. yoelii, is highly dependent upon host genes. Only 2 of 10 congenic strains of mice were strongly protected, and only one of these was protected by CD8+ T cells.

MATERIALS AND METHODS

Mice. BALB/cJ, B10.BR, B10.D2, and C57BL/10 mice were purchased from The Jackson Laboratories, Bar Harbor, ME. Other strains of mice were obtained from BIOCON, Inc., Rockville, MD. Dr. David Sachs, Immunology Branch, National Cancer Institute, National Institute of Health, provided us with BALB/c, BALB.B, and BALB.K mice backcrossed to a common BALB within six generations. Mice were between 6 wk and 6 mo old at the start of the experiments. All experiments were run in compliance with the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, DHHS Publication (NIH) 86-23, Bethesda, MD.

Parasites. P. yoelii 17X(NL) sporozoites from an uncloned line were grown and harvested as previously described (3). For some experiments a cloned parasite, P. yoelii clone 1.1, derived from this line was used. Cloning was by injection of sporozoites at limiting dilution into naive mice. Sporozoites were then produced, and the limit dilution cloning repeated. The chance that the resulting clone derived from more than a single infecting organism was less than 1%.

Serology. Mice were bled for serum before challenge. This was analyzed for antisporeozoite antibody by immunofluorescent assay to air dried sporozoites (6) and by ELISA (7). Using as capture Ag an 18 amino acid synthetic peptide (Gln-Gly-Pro-Gly-Ala-Pro)x3 whose sequence derives from the major repeat of the P. yoelii circumsporozoite protein.

T cell depletions. T cells were depleted from immune animals and analyzed by methods previously described (3). Briefly, mAb to CD8 and CD4 lymphocyte surface molecules were injected into mice. Their peripheral blood lymphocytes and spleen cells were then examined by flow microfluorometry to determine the relative numbers of T cells having CD8+ and CD4+ surface staining. Parasitemia. Thin blood films were made daily starting on the 3rd day after parasite challenge. Blood films were Giemsa stained and 50 oil-immersion fields were scanned for parasites. Mice were considered protected if no parasites were seen by day 14 after challenge.

Statistical methods. Analysis of the effect of H-2 alleles was performed using a Brandt-Snedecor decomposition of a x2 statistic (8), and the p values were adjusted to correct for the multiple comparison nature of this test. Interaction between H-2 type and background genotypes was accomplished using a log-linear model (9).

Protocol for immunization and challenge. Sporozoites for immunization received 12,000 rad from a Cs-137 source. Mice were immunized via the tail vein and injections were made at 2- to 4-wk inter-

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3 Abbreviations used in this paper: CS circumsporozoite, IFA immunofluorescent assay.
vals, with 75,000 sporozoites for the initial dose, and 25,000 sporozoites for each of three subsequent doses. Two weeks after the last dose the animals were challenged i.v. with live sporozoites, and followed with thin blood smears for 14 days. For some challenges, the infectivity of the sporozoites was monitored by serially diluting the parasites and injecting aliquots into normal mice. The number of parasites needed to infect 50% of normal animals was calculated, and this ID$_{50}$ was used to quantify the challenge dose. To confirm the stage specificity of immunity, in certain experiments mice that did not develop blood stage infections after sporozoite challenge were injected with $10^6$ P. yoelii blood stage parasites and monitored for blood stage infections.

**RESULTS**

Strains of congenic mice were immunized with irradiated P. yoelii sporozoites and there was great variability in the fraction of animals protected against challenge with 5000 sporozoites (Table I). Among the BALB congenics, BALB/c (H-2d) was highly protected but other H-2 alleles were associated with very poor protection. Within the series of B10 congenics only B10.Q (H-2q) was strongly protected. Thus, H-2 linked genetic control of immunity was found but differed depending on the non-H-2 background. This suggests the existence of more than one mechanism of protection. In every poorly protected strain, a majority of immunized mice could be infected with only 200 sporozoites (data not shown), whereas protected strains were resistant to 5000 sporozoites. Therefore, the difference between protected and nonprotected strains is more than a subtle difference in threshold. Titers of serum antibody to sporozoites did not correlate, whereas protected strains were resistant to 5000 sporozoites. Nevertheless, we challenged unimmunized mice of strongly and weakly protected strains with serially diluted sporozoites to see if infectivity differences could be the cause of the genetic variability (10). The ID$_{50}$ for intravenously injected sporozoites was the same in BALB/c, B10.D2, and B10.Q strains (data not shown).

**DISCUSSION**

This is the first report of genetic control of immunity to preerythrocytic stages of malaria. However, many host-parasite relationships, including the blood stage malaria infections, are modulated by host genes (11-14). Preerythrocytic immunity in P. yoelii is controlled by genes inside and outside the H-2 region. Genes within the H-2 region are critical. BALB/c (H-2d) mice are strongly protected but BALB strains carrying H-2b or H-2k alleles are not. Similarly, among the B10 congenics strong responses are associated only with H-2q alleles. Genes outside the H-2 region also exert important effects. This is seen comparing immunizations in BALB/c and B10.D2 mice that both have H-2d alleles. The first is strongly protected and the second only weakly so. These non-H-2 genes may be akin to the Lsh gene that maps outside the H-2 region, and that determines the rodent response to other intracellular pathogens including *Leishmania, Salmonella,* and *Mycobacteria* (15). The Lsh gene appears to exert its control by altering macrophage function (16). Overall, effects of H-2 and non-H-2 genes allowed only 2 of 10 strains to be strongly protected by irradiated *P. yoelii* sporozoites.

The results imply that there are at least two distinct mechanisms of protection, neither correlated with antibody, both under H-2-linked Ir gene control, and yet only

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<th>H-2</th>
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<th>Total</th>
<th>% (infected)</th>
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<tr>
<td></td>
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<tr>
<td>d</td>
<td>BALB/c</td>
<td>2/48</td>
<td>(4)</td>
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<td>b</td>
<td>BALB.b</td>
<td>13/16</td>
<td>(81)</td>
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<tr>
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<td>13/15</td>
<td>(86)</td>
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<tr>
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<td>B10.M</td>
<td>3/3</td>
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<td>q</td>
<td>B10.KII</td>
<td>4/4</td>
<td>(100)</td>
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<td>B10.Q</td>
<td>4/21</td>
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*a Table I is compiled from a series of experiments immunizing congenic mice with *P. yoelii* sporozoites. Both the uncloned *P. yoelii 17XNL* and the parasite clone 1.1 gave similar results, and the data have been combined. All animals were immunized with four doses of sporozoites as described in the protocol, and challenged with 5000 sporozoites. $\chi^2$ analysis shows that protection in BALB/c is significantly different from BALB/B ($p < 0.0001$) or BALB.K ($p < 0.0001$), B10.Q ($p < 0.001$), and B10.BR ($p < 0.01$). B10.Q ($p < 0.01$), and B10.BR ($p < 0.01$) are significantly different from all other B10 strains. In addition, there is a significant interaction effect between H-2 alleles and background genes ($p < 0.01$).
one dependent on CD8+ T cells. Non-H-2 genes determine which effector mechanism is activated. BALB/c mice are no longer immune to sporozoites once they are depleted of CD8+ T cells [3]. In contrast, an equivalent depletion of more than 95% of CD8+ T cells did not affect immunity in the protected B10 congenic strains (Table III). It seems unlikely that the residual CD8+ T cells are protecting these mice against large sporozoite challenges. Inasmuch as antibodies to sporozoites do not correlate with protection, we infer another immune effector mechanism, which is presumably T cell dependent because of its H-2-linked control. CD4+ T cells may be involved in this mechanism, either directly as cytotoxic cells [17, 18] or by secreting lymphokines.

Using B10 congenic mice from the same facility, Hoffman et al. (5) immunized with irradiated P. berghei sporozoites and found all strains to be strongly protected. These different patterns of response to two closely related parasites, Irl gene controlled in P. yoelii and not in P. berghei, seem paradoxical. We can only speculate on the causes.

The most likely explanation for the genetic control in P. yoelii is that only a few T cell epitopes control the important protective immune mechanisms. Congenic strains unresponsive to these epitopes are poorly protected. This is consistent with the paucity of T cell epitopes on other malaria antigens [19]. The wider immunogenicity in P. berghei could be due to presentation of a larger number of T cell epitopes [20], or recognition of a single epitope by many H-2 alleles [21]. Alternatively, a non-T cell effector mechanism might be protecting the B10 congenics against P. berghei, hiding the Irl gene control of cellular immunity. Finally, P. yoelii might have a genetically restricted suppressor mechanism [22] which is absent in P. berghei. At present there is little evidence to support any of these hypotheses.

It is not known whether immunity to sporozoites of the human malaria is genetically controlled. Our data with P. yoelii show that genetic control can occur in sporozoite immunization. If some or all of the human parasites follow the P. yoelii model, this will be another hurdle to overcome in developing antisporeozoite malaria vaccines.

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REFERENCES


| TABLE II
| Serologic analysis of congenic strains immunized with P. yoelii sporozoites |
| Protectiona | ELISA Titerb (SD) | IFI Titerc |
| BALB/c | + | 5.9 (3.0) | 256 |
| BALB.B | - | 7.2 (0.8) | 512 |
| BALB.R | + | 7.5 (1.1) | 1024 |
| B10.D2 | - | 7.1 (2.2) | ND |
| B10 | + | 7.8 (1.2) | ND |
| B10.BR | + | 8.5 (0.2) | ND |
| B10.Q | + | 7.2 (0.8) | 2048 |

a +, >90% protected; +, 20 to 80% protected; -, <20% protected.
b Capture Ag for ELISA was a synthetic peptide of 18 amino acids corresponding to three copies of the P. yoelii CS repeat sequence Glu-Gly-Pro-Gly-Ala-Pro. Titer values are expressed as [log2] of the serum dilution giving half maximal activity. Data is for serum from individual mice, four to eight samples per strain.
c IFI titer of pooled sera against air dried P. yoelii sporozoites.

| TABLE III
| Effect of depleting CD8+ T cells on protection in sporozoite-immunized animals |
| Immune + | Immune + | Normal |
| Anti-CD8 | Anti-CD8 | Control mAb |
| BALB/c | 10/10 | 0/10 | 1/5 |
| B10.BR | 4/9 | 2/9 | 5/5 |
| B10.Q | 2/8 | 1/8 | 6/6 |

a Data are presented as (mice infected/mice challenged). Mice were immunized with irradiated sporozoites and challenged with 5000 sporozoites. Protected animals were either injected with an anti-CD8 mAb or a control mAb of the same isotype [3], resulting in a depletion of 95% of the CD8+ T cells. All immunized animals and normal controls were then rechallenged with 5000 sporozoites, and blood smears followed for 14 days. BALB/c mice were no longer immune after CD8 depletion. However, B10.BR and B10.Q mice behaved differently from BALB/c (p < 0.02), and were still immune.
