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Genetic restriction of protective immunity to *Plasmodium yoelii* sporozoites

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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 which did not develop blood-stage infections. Genes both within and outside the H-2 region affected the degree of protection. Immunity did not correlate with anti-sporozoite antibody levels.* In vivo *depletion of CD8⁺ T cells did not alter immunity in two of three congenic strains, implying the existence of a novel mechanism of cell-mediated immunity.*

Introduction

The pre-erythrocytic stages of malaria are an attractive target for vaccine development. Immunity to sporozoites or hepatic stages, if completely effective, would protect an individual from developing a blood-stage parasitaemia with its debilitating and sometimes lethal symptoms. Immunization with irradiated sporozoites can completely protect against subsequent sporozoite challenge (*1*), and studies of pre-erythrocytic immunity are based on this model. Both antibodies and cell-mediated immunity have been shown to have roles in protecting against sporozoite challenge. Monoclonal antibodies to the circumsporozoite protein of both *Plasmodium berghei* (*2*) and *P. yoelii* (Y. Charoenvit, personal communication) can protect mice against high doses of sporozoites. Recently, CD8⁺ T cells stimulated by irradiated sporozoites have been identified as crucially important in protecting mice against these same two malarias (*3*, *4*). A subunit vaccine could potentially induce both of these effector immune mechanisms. However, for a vaccine to be effective it must induce a response in a large proportion of the population. A vaccine which induces strong responses only in genetically defined subgroups is of little practical use.

Recently, Hoffman et al. (*5*) immunized congenic mice with *P. berghei* sporozoites to assess whether different alleles of the major histocompatibility complex (called H-2 in the mouse) affected pre-erythrocytic immunity. All their congenic strains were protected from blood-stage infection, and it was concluded that immunity was not genetically restricted. This augured well for the development of a subunit vaccine which was genetically unrestricted as well. However, we have discovered that irradiated sporozoites of another rodent malaria, *P. yoelii*, induce strong protective immunity in only a few strains of congenic mice (*6*). We will discuss our findings, and speculate on the causes of this difference between responses to two closely related parasites.

Materials and methods

Briefly, 10 strains of congenic mice were immunized with irradiated sporozoites of *Plasmodium yoelii* 17X (NL) (*6*). Animals were given an initial dose of 75 000 sporozoites, followed by 3 booster doses of 5000 sporozoites at two to four week intervals. Two weeks after the last dose, animals were challenged with 5000 infectious sporozoites, and were monitored for 14 days to see if blood-stage infection occurred. Protected animals from some strains were then injected with monoclonal antibody to the lymphocyte CD8 molecule, which depleted their CD8⁺ T cells, and rechallenged with 5000 sporozoites.

Results

Of the 10 congenic strains of mice immunized with *P. yoelii* sporozoites, only two had a high percentage of animals which did not develop blood-stage malaria after challenge with 5000 sporozoites (Table 1). BALB/c and B10.Q mice were strongly protected, while B10.BR mice had an intermediate level of immunity. In the 7 remaining strains of mice less than 20% of the animals remained free of a blood-stage infection. With this high-dose challenge normal control animals of all strains were infected, so some immunity was induced by irradiated sporozoites in all strains. However, in only the 3 congenic strains mentioned were responses adequate to protect more than

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Table 1: **Congenic mice developing a blood-stage infection after immunization and challenge with *P. yoelii* sporozoites[a]**

H-2	Strain[b]	No. infected/total	Strain[c]	No. infected/total
d	BALB/c	2/48 (4)[d]	B10.D2	16/18 (89)[d]
b	BALB.B	13/16 (81)	B10	18/21 (85)
k	BALB.K	13/15 (86)	B10.BR[b]	33/48 (69)
s			B10.S	3/3 (100)
f			B10.M	3/3 (100)
r			B10.R III	4/4 (100)
q			B10.Q[b]	4/21 (19)

[a] This table is compiled from a series of experiments and is reproduced with permission (*6*).
[b] Chi-squared analysis shows that protection in BALB/c is significantly different ($P < .0001$) from BALB.B or BALB.K ($P < .0001$), and that B10.Q ($P < .001$) and B10.BR ($P < .01$) are significantly different from all other B10 strains.
[c] In addition, there is a significant interaction effect between H-2 alleles and background genes ($P < .01$).
[d] Figures in parentheses are the percentages infected.

a small percentage of animals. Anti-sporozoite antibodies did not correlate with protection (Table 2).

It was possible that the strain differences were not due to immune phenomena but were the result of differing susceptibility to sporozoite infection in different congenic strains (*7*). Normal mice of all 10 strains invariably came down with blood-stage infections after challenge with 200 or 5000 sporozoites. Nevertheless, on a single day we compared the number of sporozoites needed to infect normal mice of BALB/c, B10.D2, and B10.Q strains. Sporozoites were serially diluted, and injected into groups of animals. There was no difference between strains in the number of sporozoites needed to infect 50% of normal mice (infectious dose 50) (data not shown).

The congenic strains used for these experiments had been maintained separately for many generations. It was possible that the variable responses within the BALB or B10 congenics were due to genetic drift between background genes, and not to their H-2 allelic differences. To test this possibility, we immunized BALB/chk, BALB.B and BALB.K mice which had been recently bred onto a common BALB background and then reisolated. When challenged, we obtained the same results as before, all BALB/c mice being completely protected while most of the BALB.B and BALB.K animals developed blood-stage infections (data not shown). Thus, variable immunity was indeed due to H-2 differences.

$CD8^+$ T cells are important for pre-erythrocytic immunity to *P. yoelii* in BALB/c mice. When these cells are removed by *in vivo* injection of anti-CD8 monoclonal antibody, immune BALB/c mice become susceptible to sporozoite challenge (*3*). We injected anti-CD8 monoclonal antibody into protected mice of BALB/c, B10.Q, and B10.BR strains and were able to remove more than 95% of their $CD8^+$ T cells as determined by flow microfluorometry (*3*). Upon rechallenge, BALB/c mice had lost their immunity as before (Table 3). However, both B10.Q and B10.BR were still protected even though they lacked this T-cell subgroup. These animals were transfused with blood-stage *P. yoelii* parasites and came down with normal blood-stage infections, confirming that their immunity was to pre-erythrocytic forms.

Table 2: **Serologic analysis of congenic strains immunized with *P. yoelii* sporozoites[a]**

Strain	Protection[b]	ELISA titre[c]	IFA titre[d]
BALB/c	+	5.9 (3.0)[e]	256
BALB.B	−	7.2 (0.8)	512
BALB.K	−	7.3 (1.8)	1024
B10.D2	−	7.5 (1.1)	2048
B10	−	7.8 (1.2)	ND
B10.BR	+/−	8.5 (0.2)	ND
B10.Q	+	7.2 (0.8)	2048

[a] Table is reproduced with permission (*6*).
[b] + = >80% protected, +/− = 20–80% protected, − = <20% protected.
[c] Capture antigen for ELISA was a synthetic peptide of 18 amino acids corresponding to three copies of the *P. yoelii* CS repeat sequence Gln-Gly-Pro-Gly-Ala-Pro. Titres are expressed as $-\log_2$ of the serum dilution giving half maximal activity. Data are for serum from individual mice, 4 to 8 samples per strain.
[d] IFA titre of pooled sera against air-dried *P. yoelii* sporozoites.
[e] Figures in parentheses are S.D.

Table 3: **The effect of depleting $CD8^+$ T cells on protection in sporozoite-immunized animals[a]**

Strain	Immune + anti-CD8[b]	Immune + control MAb[b]	Normal mice
BALB/c	10/10	0/10	5/5
B10.BR	4/9	2/9	5/5
B10.Q	2/8	1/8	6/6

[a] Data are presented as No. infected/No. challenged. Table is reproduced with permission (*6*).
[b] 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.

Discussion

Genetic control of host–parasite interactions is well described in many different model systems (*8*). Even the erythrocytic stages of malaria show complex immunogenetic regulation (*9–11*). Therefore it should not be surprising to discover that pre-erythrocytic stages of malaria are also under control of host genes. In our studies of *P. yoelii*, we have found important effects of host genes both inside and outside the H-2 region. BALB/c (H-2^d) mice are strongly protected by sporozoite immunization, while BALB.B (H-2^b) and BALB.K (H-2^k) mice are weakly protected. Control by the H-2 locus also occurs on the C57BL/10 background, where B10.q (H-2^q) mice are strongly protected; B10.BR (H-2^k) mice show intermediate protection but other alleles are associated with weak protective responses. Genes outside the H-2 region can modulate the effect of the immune response genes. BALB/c and B10.D2 mice both carry H-2^d alleles but the first is strongly protected while the second is weakly protected. This difference must be due to the effects of non-H-2 genes. This type of genetic control might be similar to that exhibited by the *Lsh* gene which affects macrophage function (*12*). Via this mechanism *Lsh* controls strong or weak responses to many intracellular pathogens, including leishmania, salmonella, and mycobacteria (*13*). The combination of control by H-2 linked and background genes results in a severe limitation on the response to sporozoite challenge. Only two of 10 strains we tested were strongly protected by sporozoite immunization.

There appear to be two distinct, cell-mediated effector mechanisms which are under independent control by host genetic factors. BALB/c mice are protected by a mechanism critically dependent on $CD8^+$ T cells. Removing these cells leaves the mice unprotected, indicating that any other effector mechanisms in BALB/c mice are inadequate. However, an equivalent reduction in $CD8^+$ T cells in immune B10.Q or B10.BR mice does not alter protection in these animals. Either the few residual $CD8^+$ T cells are sufficient to protect these animals against a large sporozoite challenge, or another immune mechanism must be protecting these mice. This protective mechanism does not correlate with anti-sporozoite antibody levels, and is probably T-cell dependent as it is H-2 restricted. It is possible that $CD4^+$ T cells might be protecting these animals either by direct cytotoxic activity (*14*) or by the release of lymphokines.

Hoffman et al. (*5*) have immunized the C57BL/10 congenic mouse strains with irradiated *P. berghei* sporozoites. They found all strains to be strongly protected against sporozoite challenge. *P. yoelii* and *P. berghei* are closely related species, so it seems paradoxical that one parasite should be genetically restricted while the other is not.

There are at least three possible causes for this difference. We believe the most likely explanation for genetic restriction in *P. yoelii* is that very few T-cell epitopes control the protective immune responses. These epitopes would be recognized by products of only a few H-2 alleles, and mice from congenic strains carrying these alleles would be the only ones strongly protected. It is possible that *P. berghei* sporozoites expose more T epitopes to the host immune system than do *P. yoelii* sporozoites. *P. berghei* sporozoites are not very infective to mice after dissection. In Hoffman's study (*5*), 10 000 *P. berghei* sporozoites did not always infect all of his normal control animals. In contrast, dissected *P. yoelii* sporozoites are highly infectious, with 200 sporozoites reliably infecting all normal mice. If sporozoites do not readily invade mouse hepatocytes, they may be more susceptible to antibody attack and more apt to lodge in other parts of the reticulo-endothelial system. In this way *P. berghei* antigens might be processed by different cells than those of *P. yoelii* sporozoites. A wider variety of epitopes might be presented to the host's immune system, including internal antigens which may contain more T-cell epitopes (*15*). Thus in *P. berghei*, poor infectivity of sporozoites would lead to presentation of a wide range of parasite antigens, and mice carrying all H-2 alleles would be able to respond to some T-cell epitopes. If most *P. yoelii* sporozoites reach the hepatocyte, their antigens would be presented to the immune system in a limited manner, and recognition of these few epitopes might determine which strains of mice are high responders.

A second hypothesis to explain the lack of genetic restriction to *P. berghei* sporozoites concerns the protective role of antibody. Although passive transfer of anti-CS monoclonal antibodies can protect against either malaria, immune serum seems to be more active against sporozoites of *P. berghei* than against those of

Table 4: **Comparison of genetic effects in *P. yoelii* and *P. berghei*: percentage developing a blood-stage infection after homologous sporozoite immunization and challenge**

Strain	*P. yoelii*	*P. berghei*[a]
B10.D2	89	0
B10	85	0
B10.BR	69	22
B10.S	100	22
B10.M	100	10
B10.A III	100	0
B10.Q	19	0

[a] Data from Hoffman et al. (*5*). In these experiments, mice were immunized with four doses of irradiated *P. berghei* sporozoites and challenged with 10^4 *P. berghei* sporozoites.

P. yoelii. Egan and Zavala et al. (*16*) and Egan et al. (*17*) were able to protect mice against 500–1500 *P. berghei* sporozoites using synthetic vaccines designed to induce anti-sporozoite antibodies. However, Lal et al. (*18*) were unable to protect mice against *P. yoelii* using a similar immunization strategy. Also, high-titred anti-sporozoite antibody cannot protect mice against 200 *P. yoelii* sporozoites in the absence of $CD8^+$ T cells (*19*). The combination of poorly infective *P. berghei* sporozoites and protective serum antibodies could have masked genetic control of other immune mechanisms in the *P. berghei* congenic study.

The third explanation for genetic restriction in *P. yoelii* would be the existence of a suppressor factor. Suppressor epitopes are known to exist in model protein systems (*20*). If such epitopes existed in *P. yoelii*, congenic strains recognizing them would not produce vigorous T-cell responses. The strongly protected strains of mice would not carry alleles responsive to the suppressor epitopes. This would explain the heterogeneity of immune mechanisms in the different strains strongly protected against *P. yoelii* sporozoites. Perhaps in both *P. berghei* and *P. yoelii* there are many potentially protective antigens activating different immune mechanisms. In *P. berghei*, where no suppressive mechanism is active, the result is that all congenic strains are protected but by different effector mechanisms. In *P. yoelii*, this same range of antigens exists, but suppression damps the protective responses in all but a few strains. However, these no-suppressed strains may still be responding to different antigens with different immune responses. Thus BALB/c mice are protected via $CD8^+$ T cells but B10.Q and B10.BR are protected by a different mechanism.

There is little evidence to help us choose between these three possible explanations of genetic restriction to *P. yoelii* sporozoites. What is clear is that mouse–parasite pairings produce a variety of immune responses. There is not one unified rodent malaria model, but many different models. Perhaps certain of these mouse–parasite pairs mimic one or another of the human malarias but these homologies remain to be sorted out.

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References

1. **Nussenzweig, R.S. et al.** Protective immunity produced by the injection of X-irradiated sporozoites of *Plasmodium berghei*. *Nature*, **216**: 160–162 (1967).
2. **Potocnjak, P. et al.** Monovalent fragments (Fab) of monoclonal antibodies to a sporozoite antigen (Pb 44) protect mice against malaria infection. *J. exp. med.*, **151**: 1504–1513 (1980).
3. **Weiss, W.R. et al.** $CD8^+$ T cells (cytotoxic/suppressors) are required for protection in mice immunized with malaria sporozoites. *Proc. Natl Acad. Sci., USA*, **85**: 573–576 (1988).
4. **Schofield, L. et al.** Gamma-interferon, $CD8^+$ T cells and antibodies required for immunity to malaria sporozoites. *Nature*, **330**: 664–666 (1987).
5. **Hoffman, S. et al.** Immune-response gene regulation of immunity to *Plasmodium berghei* sporozoites and circumsporozoite protein vaccines: overcoming genetic restriction with whole organism and subunit vaccines. *J. immunol.*, **142**: 3581–3584 (1989).
6. **Weiss, W.R. et al.** Genetic restriction of immunity to *Plasmodium yoelii* sporozoites. Submitted.
7. **Most, H. et al.** Susceptibility of genetically standardized (JAX) mouse strains to sporozoite- and blood-induced *Plasmodium berghei* infections. *Mil. Med.* **131** (suppl.): 915–918 (1966).
8. **Skamene, E.**, ed. *Genetic control of natural resistance to infection and malignancy*. New York, Alan R. Liss, 1985.
9. **Stevenson, M.M.** Genetic control of resistance in mice to *Plasmodium chabaudi*. In: Skamene, E., ed. *Genetic control of natural resistance to infection and malignancy*. New York, Alan R. Liss, 1985, pp. 531–543.
10. **Sayles, P.C. & Wassom, D.L.** Immunoregulation in murine malaria. *J. immunol.*, **141**: 241–248 (1988).
11. **Greenberg, J. & Kendrick, L.P.** Parasitemia and survival in inbred strains of mice infected with *Plasmodium berghei*. *J. parasitol.*, **43**: 413–419 (1956).
12. **Crocker, P.R. et al.** Expression of the natural resistance gene *Lsh* in resident liver macrophages. *Infect. immun.*, **43**: 1033–1040 (1984).
13. **Bradley, D.J.** Regulation of *Leishmania* populations within the host. II. Genetic control of acute susceptibility of mice to *Leishmania donovani* infection. *Clin. exp. immunol.*, **30**: 130–140 (1977).
14. **Ozaki, S. et al.** Cloned protein antigen-specific Ia-restricted T cells with both helper and cytolytic activities: mechanisms of activation and killing. *Cell immunol.*, **105**: 301–316 (1986).
15. **Dontfraid, F. et al.** Human and murine CD4 T-cell epitopes map to the same region of the malaria circumsporozoite protein: limited immunogenicity of sporozoites and circumsporozoite protein. *Molec. biol. med.*, **5**: 185–196 (1988).
16. **Zavala, F.** Synthetic peptide vaccine confers protection against murine malaria. *J. exp. med.*, **166**: 1591–1596 (1987).
17. **Egan, J.E. et al.** Efficacy of murine malaria sporozoite vaccines: implications for human vaccine development. *Science*, **236**: 453–456 (1987).
18. **Lal, A.A. et al.** *In vivo* testing of subunit vaccines

against malaria sporozoites using a rodent system. *Proc. Natl Acad. Sci., USA*, **84**: 8647–8651 (1987).

19. **Sedegah, M. et al.** Use of a vaccinia construct expressing the circumsporozoite protein in the analysis of protective immunity to *Plasmodium yoelii*. *Tech. adv. vac. devel.*, 295–309 (1988).

20. **Wicker, L.S. et al.** Immunodominant protein epitopes. I. Induction of suppression to hen egg-white lysozyme is obliterated by removal of the first three *N*-terminal amino acids. *Europ. j immunol.*, **14**: 442–447 (1984).