NOTES

A Single Gene Determines Rapid Expulsion of *Trichinella spiralis* in Mice

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In rats and some inbred mouse strains, one immune response, rapid expulsion, confers up to 95% protection against a challenge infection with *Trichinella spiralis*. Strain analysis in mice has shown that only three inbred strains, all originating from Swiss-line mice at the National Institutes of Health, Bethesda, Md., express rapid expulsion. Crosses between responder strain mice (NFR/N) and nonresponders (C3H/HeJ or B10·BR) have indicated that rapid expulsion is dominant and autosomal (Bell et al., Exp. Parasitol. 53:301–314, 1982). In this study a segregation analysis of rapid expulsion in the F2 and backcross conformed to the Mendelian ratios expected of a single gene. This gene was not linked to the major histocompatibility complex (MHC) (chromosome 17) or the gene for albinism (c/c locus on chromosome 7). This locus has not previously been identified as conferring resistance to any infectious agent, and we have therefore designated the gene Ihe-1 (intestinal helminth expulsion 1).

Despite the prevalence of human intestinal helminths (1 billion infected with Ascaris, 700 million with hookworm) (21) and the economic costs of these parasites in livestock management, our knowledge of immunity to intestinal helminths is rudimentary. Rapid expulsion has only recently been recognized as the single most effective intestinal immune response expressed by rodents against several species of parasitic helminths (1, 2, 6, 7–11). All rat strains examined so far display rapid expulsion of a challenge infection with *Trichinella spiralis*, but only three inbred murine strains are recognized as responders for this response. These three are the NFR/N and NFS/N strains (5), both originating in 1972 at the National Institutes of Health (NIH), and a strain inbred in England and designated NIH, that also originated from NIH outbred Swiss mice (15). Inbred mouse strains demonstrated to be nonresponders for rapid expulsion include Swiss-derived SJL and SWR strains (13) as well as BALB/c, CBA, A, C3H, C57BL/6 and substrains, DBA/1 and DBA/2 (5). The existence of this strain variation provides an opportunity to analyze the genetic mechanisms controlling rapid expulsion. Previous experiments from this laboratory have shown that rapid expulsion is a dominant trait which is not sex linked (5). This strain distribution pattern and the behavior of F1 crosses suggested that a single dominant gene determined the expression of rapid expulsion. We present here evidence showing that rapid expulsion is controlled by a single gene unlinked to the major histocompatibility complex (MHC) or the coat color locus determining albinism.

The experiments involved crossing mice of the NFR/N strain (responder) to nonresponder mice of both the inbred C3H/HeJ and the congenic inbred B10·BR lines. The C3H/HeJ and B10·BR strains showed pronounced, genetically determined differences in their ability to eliminate primary *T. spiralis* infections; the C3H/HeJ strain rejects an infection with 400 *T. spiralis* in 14 days, whereas B10·BR mice reject a primary infection with *T. spiralis* in 21 to 24 days (5). Both strains differ from the NFR/N strain in coat color (NFR/N = albino, C3H/HeJ = agouti, and B10·BR = black) and in MHC haplotype, where NFR/N = H-2* (K and D) as typed in this laboratory (R. G. Bell, unpublished results), and both C3H/HeJ and B10·BR are H-2*. F1 mice from the cross of either NFR × B10·BR or NFR × C3H/HeJ display strong rapid expulsion resulting in the elimination of between 85 and 95% of the challenge infection within 24 h (worm count: C3H/NFR F1, 46 ± 46 control; B10·BR × NFR F1, 36 ± 31 immune and 372 ± 65 control).

Backcrosses and intercrosses of NFR/N to both the B10·BR and C3H/HeJ strains were examined to determine whether the genetic component establishing time of rejection in a primary infection could also influence the rapid expulsion response. The upper limit of rapid expulsion was defined at the lower 5% fiducial limits of the mean worm burden of immune nonresponder mice or nonimmune responder mice (mean – 2 standard deviations). Mice were immunized by a standard protocol (Fig. 1), and statistical analyses were performed assuming that the expression of rapid expulsion was controlled by a single dominant gene (for a more complete description of statistical methodology, see reference 18). Backcrosses to the C3H/HeJ or the BIO·BR strain segregated in accordance with the predicted ratio of 1:1 (Table 1). Segregation of the intercross of each F1 hybrid (NFR/C3H × NFR/C3H) (NFR/BIO·BR × NFR/BIO·BR) again resulted in a division of responders and nonresponders (3:1) that was consistent with the single dominant gene hypothesis (Table 1). No difference in the strength of expression of rapid expulsion, as measured by worm burden, could be ascribed to the background effects of the strong C3H/HeJ or weak B10·BR genes influencing time of rejection of adults in the primary infection.

Genes linked to the MHC have been shown to influence resistance to *T. spiralis* in mice (20, 22). Therefore, linkage of rapid expulsion to the MHC was assessed by using the C3H/HeJ (H-2*) nonresponder as one parent. Tissue typing of NFR/N mice and their crosses with the C3H/HeJ strain
was performed on mesenteric lymph node cells. At the time of intestine removal, the mesenteric lymph node was also removed, and a single cell suspension was prepared by gently rubbing the node through a 40-mesh nickel screen. The cells were washed twice and resuspended at 107 cells per ml in Dulbecco phosphate-buffered saline. To detect H-2 Kq present on the NFR/N parental mice, 50 µl of cell suspension was incubated with mouse antiserum D-17 (H1 × C × AKR × M) anti-DBA/1, obtained from J. G. Ray, Transplantation Immunology Branch, Collaborative Research, National Institute of Allergy and Infectious Diseases) at a final dilution of 1/20 for 60 min on ice. Antiserum D-17 (K1 D1 × K2 D2) anti Kq was cytotoxic for NFR/N lymph node cells but unreactive with C3H/HeJ or B10 × BR (H-2b) lymph node cells. Cells were washed once and resuspended in 50 µl of rabbit complement (Low-Tox rabbit complement; Cedarlane Laboratories) diluted 1/15 and incubated for 45 min at 37°C. Cells were placed in ice, and trypan blue was added before microscopic quantitation of killed and live cells. Percent cytotoxicity was estimated by the formula: 1 – [number of viable cells after treatment with H-2 specific serum plus complement]/[number of viable cells after treatment with normal serum plus complement)] × 100 and compared with parental controls (NFR/C3H and C3H). Tissue typing was performed “blind” on the day of harvest, whereas worm counts were done by another investigator on the day after intestinal harvest. Typing of the backcross progeny of the F1 × F1 nonresponder (NFR/C3H × C3H/HeJ) showed 8 rapid expulsion-positive mice to 15 nonresponder mice (χ² = 2.13; not significant) and 10 mice that were H-2k versus 12 H-2b (χ² = 0.48; not significant). Of the observed 10 H-2k mice, 4 displayed rapid expulsion, whereas 6 were nonresponders. If we assume that rapid expulsion was linked to the MHC, then all rapid expulsion-positive mice should also carry the responder H-2 haplotype (H-2b).

A chi-square comparison of the observed phenotypes with the expected phenotype produced a χ² of 39.6 (highly significant), and therefore no association with the MHC could be established. Similar experiments using the B10 × BR strain as the nonresponder confirmed this result.

Linkage with coat color was also assessed using segregation of the albino allele (cc, chromosome 7) of NFR/N mice with the agouti series (AA chromosome 2) of C3H/HeJ mice. Segregation of the intercross produced 14 albino (cc) offspring: of these, 9 were responders and 5 were nonresponders (χ² = 26.8; highly significant), indicating that there was no linkage to the genes for albinism. From these data we may conclude that the gene for rapid expulsion is not linked to the MHC or to the cc locus in mice, thus excluding chromosomes 7 and 17. Since these experiments were performed we have backcrossed the NFR/N gene for a further four generations to the B10 × BR line, and the segregation ratios obtained have supported the single major gene hypothesis.

Comparison of the strain distribution of rapid expulsion with the other loci known to determine resistance to infectious eucaryotic agents (Leishmania donovani, Bcg/Ity/Lsh [13, 17]; resistance to Taenia taeniaformis [12], Giardia muris [16], Trichuris muris [19], or Nematospiroides dubius [14]) shows pronounced differences in responder and nonresponder strains for each of these resistance factors as well as in responder strains for expression of rapid expulsion. This indicates that the gene for rapid expulsion is distinct from each of these loci, despite their common involvement in resistance to infectious agents. We therefore propose the terminology h-1 (intestinal helminth expulsion 1) for this locus. Earlier reports from this laboratory have shown that a complex of at least four stage-specific intestinal immune responses is involved in worm infection or inhibition of reproduction of T. spiralis (7). Rapid expulsion is the most conspicuous protective response and appears to exert its effect independently of each of the other functional responses (7). Although rapid expulsion is known to have an immunological basis (3, 4, 7), there is an additional requirement for a nonspecific intestinal component. The site of

![Graph showing the distribution of worms in immune and nonimmune groups.](image)

**Cross examined**

<table>
<thead>
<tr>
<th>Worms</th>
<th>Immune</th>
<th>Nonimmune</th>
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<tbody>
<tr>
<td>NFR/C3H</td>
<td>C3H</td>
<td>NFR/C3H × C3H</td>
</tr>
<tr>
<td>NFR/N</td>
<td>C3H</td>
<td>NFR/N</td>
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**TABLE 1. Segregation of rapid expulsion in F2 and backcross mice**

<table>
<thead>
<tr>
<th>Cross</th>
<th>Responder</th>
<th>Nonresponder</th>
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<tbody>
<tr>
<td>Backcross</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NFR/C3H × C3H</td>
<td>32</td>
<td>38</td>
</tr>
<tr>
<td>BIO × BR/NFR × BIO × BR</td>
<td>22</td>
<td>25</td>
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| Intercross | | |
| NFR/C3H × NFR/C3H | 41 | 16 |
| NFR/BIO × BR × NFR/BIO × BR | 16 | 5 |

*χ² backcross to C3H = 0.514, to BIO × BR = 0.19; χ² intercross NFR/C3H = 0.285 and NFR/BIO × BR = 0.160; none are significant. χ² was obtained by testing phenotypic segregation of rapid expulsion for conformity with the predicted single dominant gene control of rapid expulsion.*
action of Ihe-1 is at present undefined, and the gene could influence the expression of either the immunological or the nonspecific component of rapid expulsion.

It is of considerable interest that a gene with such a powerful effect on parasite rejection should be restricted in its distribution to only three related inbred mouse strains that are not commonly used experimentally. Although this pattern might indicate a recent mutation, the widespread distribution of rapid expulsion in inbred rats, as well as the existence of Ihe-1 in outbred CFW mice (also of Swiss origin), leaves open the hypothesis that the gene was fortuitously carried by the original Swiss-line mice and was randomly passed to various inbred substrains as these were developed. We are currently testing more unusual inbred strains of mice and strains recently derived from wild mice to address this question.

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LITERATURE CITED