Relapse Following Cessation of Antibiotic Therapy for Mouse Typhoid in Resistant and Susceptible Mice Infected with Salmonellae of Differing Virulence

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Relapse after cessation of ampicillin therapy in inbred mice infected with salmonellae was studied. The incidence of relapse was influenced by the following. (A) The mouse strain, with genetically susceptible animals more likely to relapse than genetically resistant animals; a possible role for the Ity gene was observed. (B) The virulence of the infecting organism, with a more virulent strain causing relapse in both resistant and susceptible mice, a less virulent strain causing relapse in neither resistant nor susceptible mice, and an intermediate strain causing relapse in susceptible mice but not in resistant mice. (C) The duration of antibiotic therapy, with prolonged treatment preventing relapse seen after short-term therapy. In all cases, ampicillin failed to clear the infection, and the mice remained carriers.

This study was undertaken to investigate the possible factors affecting the response to, and the relapse after, antimicrobial therapy for mouse typhoid. Differences in the genetically controlled host response between inbred mouse strains and differences in virulence between salmonella strains used for infection were the main factors studied.

That salmonella infections in mice are genetically controlled has long been established [1]. More recently, the nature of this control has been further elucidated and an infection can now be divided into two distinct phases, each under the control of different genes [2]. The early, acute phase of the infection is controlled by the products of a single gene or cluster of genes, designated Ity [1, 3–7]. This gene is expressed as an innate property of the macrophage [8–12], as has recently been demonstrated in vitro (C. E. H. and K. A. Harrington, unpublished observations) [13].

The later chronic phase of the infection is under polygenic control, with roles demonstrated for the Lps, xid, and other genes [14] and more recently for the H-2 gene complex [15]. The phenotypic expression of these various genotypes in terms of a mouse strain being naturally resistant or susceptible depends greatly on the virulence of the strain of Salmonella used for infection. Thus, a dose of $10^3$ of the highly virulent Salmonella enteritidis strain 5694 kills both resistant and susceptible mice in the acute phase of the infection. A similar dose of the virulent Salmonella typhimurium strain C5 kills susceptible mice in the acute phase, whereas resistant mice will control the infection and become chronic carriers. S. typhimurium strain M525, of intermediate virulence, in a similar dose kills neither susceptible nor resistant mice in the acute phase. The mice control the infection, probably by a cell-mediated immune response, and become carriers [2, 7, 8, 16–19].

In this study, mice that would normally die in the acute phase were rescued from death by ampicillin. After some days the ampicillin was discontinued and the growth rate of the bacteria in the mice was used to highlight any differences in the ability of different mouse strains to control relapses with salmonellae of differing virulence.

Materials and Methods

Mice. A/J, BALB/c, and (B10 × A/J)F, mice were all bred in this department from stock originally purchased from Olac 1976 (Blackthorn, Bicester, England). A/J breeders originally came from the Jackson Laboratories (Bar Harbor, Me) [2, 7].

Bacteria. S. typhimurium strain C5, S. typhimu­rium strain M525, and S. enteritidis strain NCTC 5694 were as described previously [2, 7]. Mice were infected iv into a tail vein with overnight broth cul-
tures (tryptic-soy broth; Difco, Detroit) that had been kept frozen at −70°C and diluted in PBS to the desired concentration. The challenge dose was adjusted for each host-parasite combination so that the bacterial load would be ~10³ when the antibiotic was begun.

Enumeration of bacteria in the liver and spleen. Livers and spleens were removed from three or four mice per point before their daily dose of ampicillin and were homogenized in 10 ml of distilled water in a Colworth Stomacher (A. J. Seward, Bury St. Edmunds, Suffolk, England). Viable counts were performed in tryptic-soy agar (Difco) by using pour plates of 1-ml samples for counts <10³ or a Colworth Droplette (A. J. Seward) for counts >10³ bacteria per organ [2, 7]. Counts are expressed as the geometric mean ± 1 SE.

Administration of antibiotic. Animals were given 20 mg of ampicillin (Sigma, Poole, Dorset, England) ip daily in 0.2-ml volumes, starting on day 3-4 of the infection.

Relapse and the carrier state. In this study, relapse is defined as a sustained and progressive increase in bacterial loads in both the liver and spleen after cessation of successful antibiotic therapy. Successful antibiotic therapy is defined as not only saving the mice from death in the acute phase of infection but also as a marked improvement in the disease in terms of the decrease in bacterial load in the liver and spleen. Bacterial carriage and the carrier state are used here in the sense used by Hobson [18], as prolonged bacterial persistence in the spleen and liver of apparently healthy mice.

Results


Eighty BALB/c mice were infected with 6.6 × 10³ organisms, and the antibiotic was begun on day 4 after infection (figure 1). Bacterial loads increased at ~1 log per day, killing all three untreated controls by day 6. All treated animals survived, with a sharp drop in bacterial numbers to 10³–10⁴ per organ by day 7.

Cessation of antibiotic on day 9 caused an immediate vigorous regrowth of the bacteria, at a rate similar to that of the preantibiotic stage. Twelve mice died between days 14 and 16. No animals survived beyond day 16.

Prolongation of antibiotic treatment until day 24 after infection ablated the relapse. During this prolonged therapy, spleen counts remained <10⁴ and sometimes dropped below 10³. Liver counts were erratic, with individual counts higher in those livers showing gross visible focal abscess-like lesions. These lesions were generally accompanied by gross splenomegaly that was not associated with high spleen counts.

Cessation of antibiotic on day 24 led to no deaths. Mean bacterial counts increased irregularly in the livers. On days 32, 35, and 38, individual liver counts

Figure 1. Eighty BALB/c mice were infected iv with 6.6 × 10³ S. typhimu- rium strain C5. Ampicillin was started on day 4 and stopped on either day 9 or day 24. Four untreated mice died on each of days 14, 15, and 16. Open symbols, treated mice; closed symbols, untreated controls; circles, livers; triangles, spleens. The arrow on the ordinate indicates the challenge dose. Reprinted with permission from the Bulletin Européen de Physiopathologie Respiratoire [12].
Thirty-six A/J mice were infected iv with 8.6 × 10^5 S. typhimurium strain C5. Ampicillin was started on day 4 and stopped on day 9. Open symbols, treated mice; closed symbols, untreated controls; circles, livers; triangles, spleens. The arrow on the ordinate indicates the challenge dose.

Bacterial counts dropped rapidly, and few focal liver lesions were seen. Cessation of therapy on day 9 was followed by an initial transitory increase in bacterial load that was then reversed. Spleen counts remained low. All mice survived the cessation of antibiotic.

Prolongation of therapy in A/J mice caused a marked decrease in bacterial loads as compared with the untreated group, but in no case were the bacteria eliminated; a carrier state [18] always remained. Similar results were seen with (B10 × A/J)F₁ mice (authors’ unpublished data).

Infections with S. typhimurium strain M525 (low virulence) and S. enteritidis strain 5694 (high virulence). The incidence of relapse was studied in susceptible mice given a less virulent organism than S. typhimurium strain C5 (strain M525) and in resistant mice given a highly virulent organism (S. enteritidis strain 5694).

Thirty-three BALB/c mice received 5.6 × 10^4 S. typhimurium strain M525 (figure 3). Bacterial counts were 10^6 on day 4, and all untreated controls were dead by day 8, before counts could be taken.

Again, starting treatment on day 4 prevented death, and bacterial counts decreased, more rapidly in the spleens than in the livers (the latter showing focal lesions by day 7). Cessation of therapy on day 9 was followed by an increase in bacterial load that were 4–5 logs higher than were the other two counts for that time point, a result accounting for the large SEs. These high counts were always associated with the large abscess-like focal lesions on the livers, whereas livers of mice with low counts had no visible focal lesions. Mean spleen counts did not increase. Individual spleen counts from mice with high liver counts were always ~1–2 logs higher than were other spleen counts for the time point. All spleen counts in this stage of infection were <10^4. Thus, prolongation of therapy in these Ity+ mice prevents the development of the fatal relapse seen after short-term therapy.

2. Resistant mice (Ity–). Thirty-six A/J mice received a challenge ~100 times that of the Ity+ mice so that a similar bacterial load would be reached in three to four days (figure 2). Bacterial growth rate was slower than that seen in Ity+ mice. All four untreated controls died.

Antibiotic was started when bacterial loads were ~10^7 in the liver and spleen. All treated mice survived. Bacterial counts dropped rapidly, and few focal liver lesions were seen.

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Discussion

These results show that, in the mouse model of typhoid, the response to antibiotic therapy and the tendency to relapse thereafter is determined to a large extent by host resistance genes, by the virulence of the infecting organism, and by the duration of treatment. The precise identity of the gene(s) involved is not clear, although these results suggest that the resistant Ity phenotype is advantageous, because Ity⁻ mice are much less prone to relapse than are Ity⁺. Ity⁻ mice behave similarly to Ity⁻ mice (authors' unpublished data). The resistant phenotype may be beneficial because it allows time for the development of an effective immune response after the second outgrowth of organisms following cessation of antibiotics. This outgrowth occurs more rapidly in Ity⁻ mice, which are overwhelmed and killed in the relapse. The confirmation of a role for Ity requires backcross analysis and studies in congenic mice possessing Ity⁻ on an otherwise Ity⁺ background, e.g., BALB/c Ity⁻ [20].

The overall control of the late phase of infection is polygenic [2]. Recent work from this laboratory shows H-2 control of the late phase of salmonella infections [15] and current results show a role for the H-2 gene complex in controlling relapse (authors' unpublished observations). Other as yet unidentified genes may also be involved. A similar situation, in which the success of chemotherapy is seen to depend on host genes, is in *Leishmania mexicana* infections in mice [21].

Relapse is also affected by the virulence of the infecting strain of salmonella. If a less-virulent strain of *Salmonella* (M525) is used in susceptible BALB/c mice, which relapse vigorously if infected with the more virulent C5 strain, they no longer relapse. This is in accordance with published data [16] that show that BALB/c mice sublethally infected with strain M525 were able to make a good delayed-type hypersensitivity response to salmonella antigen and were able to resist a challenge with the virulent C5 strain on day 8.

It is surprising that BALB/c mice seem unable to...
make a primary response to strain C5 by day 9 in this model. This is clearly not an absolute deficiency because they can eventually respond to C5 (by day 24). On the other hand, *Ity* mice, which cope adequately with a C5 infection and do not relapse, have much more difficulty in controlling a relapse caused by the more virulent *S. enteritidis* strain 5694. When antibiotic is stopped in this case, there is a systemic regrowth of the organism leading to the death of the mouse. It seems that the more virulent the organism the longer it takes the mouse to mount an efficient immunity and that resistant mice are more efficient than are susceptible mice in handling the more virulent organisms. Indeed, this model allows the study of the later stages of infections caused by virulent organisms in susceptible mice that would normally die in the acute phase.

The third major factor influencing relapse was the duration of antibiotic treatment. Mice that relapsed after a short period of treatment did not do so if treatment was continued for three weeks. However, in no case were the bacteria completely cleared.

The development of liver abscesses may play an important role in the response to ampicillin therapy in these mice. Individual excised abscesses always contained several logs more bacteria in them than in the surrounding liver tissues (D. J. M., unpublished observations). The abscess may provide a protected site into which the ampicillin cannot easily penetrate. Even in the absence of abscesses, however, in no case were the bacteria completely cleared from the reticuloendothelial system. BALB/c mice infected with strain M525 have been followed up for as long as one year. The bacterial carriage remained constant for all this time, at \(10^3-10^4\) (C. E. H., unpublished observation). The bacteria must be completely cleared to remove all possible potential for relapse; liposome-encapsulated antibiotic may be useful for this [22].

The problem of relapse after antibiotic therapy is well documented for human typhoid. Treatment with chloramphenicol was seen to reduce mortality but to increase the rate of relapse [23]. Early treatment seemed to increase the relapses [23, 24]. The period of administration of antibiotic was deemed important in preventing relapse [25-27]; more relapses were seen if antibiotic was given for eight days [28-30], although no further advantage was seen in carrying on therapy for longer than two weeks [25]. It has been suggested that permanent recovery without relapse depends on the development of effective host immunity [29, 31].

Although remaining extremely cautious, we can draw comparisons between this study and the above observations on human typhoid. In the experimental situation, antibiotic treatment of a mixed population of *Ity* and *Ity* mice would be expected to result in proportionally fewer deaths in the acute phase and in far more relapses following early cessation of therapy than would be expected in untreated controls. Prolonged therapy would further increase survival by reducing the relapse rate, beyond which point further treatment is unnecessary. Similar conclusions have been drawn from treatment of human typhoid.

It is tempting to speculate that, in addition to well-recognized factors such as bacterial dose, virulence, and period of antibiotic treatment, the genetic constitution of the host may markedly influence the efficacy of antibiotic therapy.

Clearly, the effectiveness of treatment on a genetically heterogenous population containing individuals as diverse as the mice used here would not be uniform. Evidence has been presented that suggests that genetic factors may be important in susceptibility to human typhoid [32, 33]. No markers of innate susceptibility have been identified in humans, and it remains to be seen whether the present experimental results have any bearing on human disease.

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