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Influence of H-2 genes on growth of *Mycobacterium tuberculosis* in the lungs of chronically infected mice

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SUMMARY

Mice infected by intraperitoneal injection of *Mycobacterium tuberculosis* were studied over a 23-week period. They showed progressive infection in the lung (with increasing microbial count and granuloma size) whereas viable bacillary counts remained largely stationary in the spleen and in the liver. The influence of H-2 genes on the progression of the lung infection was studied in four congenic strains of animals with B10 and three congenic strains of animals with BALB backgrounds. H-2^k mice had significantly higher bacterial counts in the lung than H-2^b mice on both B10 and BALB backgrounds, BALB. K (H-2^k) mice were also more susceptible than BALB/c (H-2^d) mice. Results with recombinant strains showed that bacillary counts and granulomatous infiltration were lower in the B10 ($K^bA^bE^-D^b$) compared with B10.A(3R) ($K^bA^bE^bD^d$) strain and in B10. A(4R) ($K^kA^kE^-D^b$) compared with B10.BR ($K^kA^kE^kD^k$) mice. This resistance to the late expansion of tuberculous infection in the lungs may be associated with the lack of an expressed I-E molecule or with the expression of the D^b molecule.

INTRODUCTION

The genetic control of susceptiblity to mycobacterial infection is generally considered to be multifactorial. In mouse experimental models, the influence of specific genes depends on the route and dose of infection and the phase of infection as well as the particular parameter of host response studied. There is evidence that the innate Bcg gene influences the early clearance of bacteria,[1] and that the IgH genes affect the extent of bacillus Calmette-Guérin (BCG) adjuvant-induced granulomatous reaction.[2] An autosomal gene designated Tbc-1 influences the mean survival time following *Mycobacterium tuberculosis* H37Rv infection.[3,4] Other less-defined non-H-2 genes have been implicated in various host reactions to mycobacteria.[5-7] The restricted impact of any individual gene is illustrated by the fact that the Bcg gene influences bacterial load following intravenous but not of respiratory infection.[8]

H-2-linked genes have been shown to influence the extent of the foot-pad granulomatous reaction between 4 and 8 weeks after local infection with *M. lepraemurium*: this effect has been greater in strains with B10[9] and lesser in strains with BALB[10] background. The resistance to local infection is T-cell mediated[11] and high responsiveness was associated with the H-2^b haplotype, which is also linked with the higher immune responses to several mycobacterial antigens.[5,12,13] H-2 genes were reported to influence mortality following massive systemic infection with virulent *M. tuberculosis*[3,4] and to act in synergy with IgH genes[14] in determining the extent of the lung granuloma response to BCG cell walls. The purpose of the present study was to investigate the organ localization of a chronic *M. tuberculosis* infection in B10 congenic strains of different H-2 haplotypes and in recombinant strains differing at various regions of the H-2 locus.

MATERIALS AND METHODS

Female, 6–8-weeks-old mice of B10 ($K^bA^bE^-D^b$), B10.A(3R) ($K^bA^bE^bD^d$), B10.A(4R) ($K^kA^kE^-D^b$), B10.BR ($K^kA^kE^kD^k$), BALB.K ($K^kA^kE^kD^k$), BALB.B ($K^bA^bE^-D^b$) and BALB/c ($K^dA^dE^dD^d$) strains were purchased from Harlem Olac Ltd (Bicester, Oxon, U.K.). Mice were infected intraperitoneally with 5×10^5 viable bacilli of *M. tuberculosis* (H37Rv), from frozen aliquots harvested from mid-log phase growth at 37° in Middlebrook 7H9 medium (Difco Laboratories, Detroit, MI). Groups of five to six mice from each strain were killed by cervical dislocation at 6, 13, 18 or 23 weeks. At post-mortem examination the lungs, spleens and livers from five to six mice were harvested and weighed. Whole-organ homogenates at serial dilutions were plated in duplicate on Middlebrook 7H11 agar (Difco) Petri dishes and colony-forming units (CFU) were counted after 20 days incubation at 37°. The results expressed as CFU/100 mg organ weight were calculated as group geometric means and compared by Student's *t*-test.

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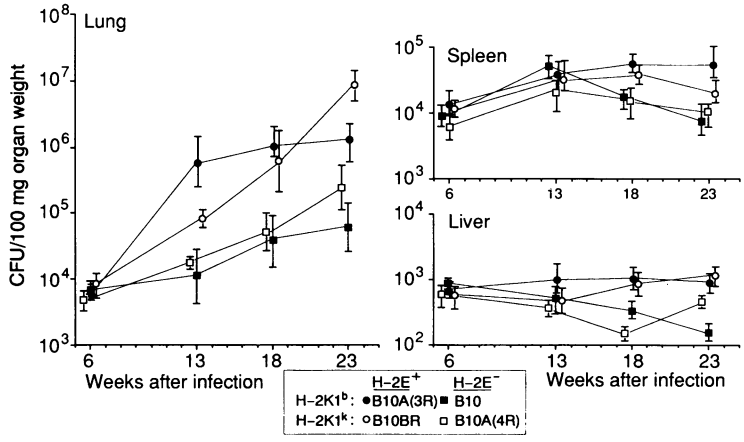

Figure 1. Influence of H-2 genes on the extent of lung infection at a late stage of infection. Mice were infected intraperitoneally with 5×10^5 viable *M. tuberculosis* H37Rv bacilli. Symbols represent geometric means ($n = 5$–6) and standard errors (vertical bars) of organ colony-forming units (CFU) at various times following infection.

Formaldehyde-fixed section of lungs harvested 6, 13 or 18 weeks post-infection were stained with haematoxylin and eosin. Granuloma fraction (=percentage of the area of section occupied by granulomatous inflammation) and the size of individual granulomata were measured using a semi-atuomatic planimeter (Imagan 2 Planimeter, Kompira Ltd, Shotts, Lanarkshire, U.K.) as previously described.[15]

RESULTS

The levels of viable H37v bacilli between 6 and 23 weeks post-infection in the livers, spleens and lungs of four B10 background strains, are shown in Fig. 1. At 6 weeks, there were no strain differences in any of the tested organs. At subsequent time intervals up to 23 weeks after infection, liver CFU declined or varied between 10^3 and 10^2 (10^2 is the lower limit of detection) but without consistent differences between the strains. Splenic CFU slightly increased from 6 to 13 weeks, but uniformly in all tested strains; subsequently, somewhat higher viable spleen counts were found in B10.A(3R) than in B10 mice at 18 and 23 weeks post-infection ($P < 0.05$, $P < 0.01$).

The progression of infection in the lungs showed pronounced strain differences. The H-2 haplotype significantly influenced the viable organism counts found in the lungs of mice infected with *M. tuberculosis* at the later stages of infection. B10 (H-2^b) mice had significantly fewer organisms than B10.BR (H-2^k) mice at 23 weeks post-infection ($P < 0.001$) (Figs 1 and 2). B10 mice also had significantly lower counts than B10.A(4R) mice ($P < 0.05$) indicating that the K and/or A loci may also influence the bacterial load to a similar extent. The more severe infection in the H-2^k haplotype was also apparent on a BALB background as BALB.K mice had significantly higher counts than BALB.B mice at 18 weeks post-infection ($P < 0.01$) (Fig. 2). BALB.K mice were also more susceptible than BALB/c (H-2^d) mice at 17 weeks post-infection ($P < 0.02$).

The genetic influence was studied in more detail by examination of two pairs of strains which carried the same H-2 allele at the K and A loci. When comparing the K^bA^b strains, B10.A(3R) (E^bD^d) mice showed at least 10 times higher CFU levels than B10 (E^-D^b) mice at 13, 18 and 23 weeks post-infection ($P < 0.001$ at all three time intervals). When comparing the K^kA^k strains,

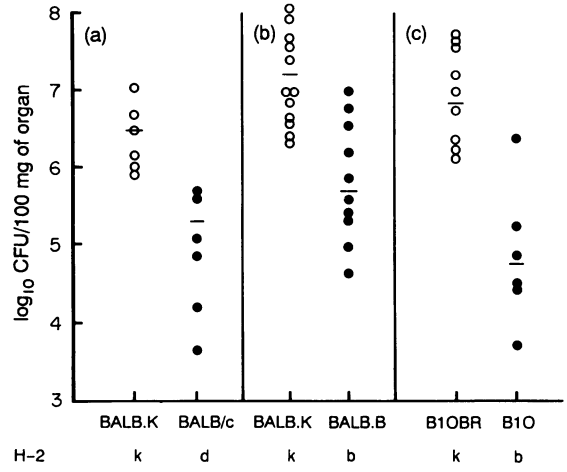

Figure 2. Influence of H-2 haplotype on the extent of late lung infection in BALB strains. Mice were infected intraperitoneally with 5×10^5 viable *M. tuberculosis* H37Rv bacilli. Viable organism counts were determined at (a) 18 weeks in Exp. 1 ($P < 0.02$), (b) 17 weeks in Exp. 2 ($P < 0.01$) and (c) 23 weeks in Exp. 3 ($P < 0.001$). Results of individual mice are shown and horizontal lines indicate the means.

significantly higher CFU counts were found in B10.BR (E^kD^k) compared with B10.A(4R) (E^-D^b) mice at 18 and 23 weeks post-infection ($P < 0.05$, $P < 0.0001$), although this difference was not significant at 13 weeks.

Morphologically, there was a progressive increase in the extent of granulomatous inflammation within the lung parenchyma between Weeks 6 and 18. For all the strains studied the granuloma fraction was maximal at Week 18. For animals with a common B10 background there were pronounced strain differences between different H-2 haplotypes (Table 1). When comparing K^bA^b strains at Week 18 post-infection, B10.A(3R), (E^bD^b) mice had a higher lung granuloma fraction than B10 (E^-D^b) mice ($P = 0.1$). Similarly, when comparing K^kA^k strains, B10.BR (E^kD^k) mice had a higher lung granuloma fraction than B10.A(4R) (E^-D^b) mice ($P = 0.1$). Neither of these two comparisons were statistically significant because the substantial inter-animal variations resulted in large standard deviations, but the

Table 1. Correlation between bacterial counts and granulomatous reaction in the lungs of infected H-2 recombinant mice

Strain of mice	Expressed genes K, A	Eβ	S	D	Log_{10} CFU /100 mg*	Granuloma fraction at Week 18 (%)*
B10	b	—	k	b	4·60±0·36	4·18±1·7
B10.A(3R)	b	b	d	d	6·04±0·31	12·41±4·4
B10.A(4R)	k	—	b	b	4·72±0·25	4·21±1·9
B10.BR	k	k	k	k	5·76±0·44	14·37±5·2

Lungs were harvested 18 weeks after infection with *M. tuberculosis* (see legend to Fig. 1).

* Results are shown as mean ± SE.

differences in size of granuloma fraction between strains clearly parallels the observed differences in CFU counts.

DISCUSSION

Classical Ir gene studies have shown that the level of immune responsiveness to different T-cell epitopes on an individual protein are influenced by class I and class II H-2 genes, but it is difficult to understand how the precise selectivity of such genes can influence susceptiblity to complicated bacterial organisms which produce multiple proteins. Alternatively, H-2-associated regulation by mechanisms which operate in respect of multiple antigens has been described.[16]

The results of the present investigation revealed an H-2-linked genetic influence on the resistance to *M. tuberculosis* in the lungs at the later stages (18–23 weeks) of infection. Mice of both B10 and BALB backgrounds showed higher susceptibility to lung infection when they carried the $H\text{-}2^k$ haplotype. Interestingly, $H\text{-}2^k$ mice infected subcutaneously with *M. lepraemurium* have also been reported to be more susceptible than $H\text{-}2^b$ mice at late stages of the infection.[10] Previously, $H\text{-}2^b$ mice have been found to have higher mortality after infection with *M. tuberculosis* than other H-2 haplotypes,[3,4] but differences with the present results may be due to technical reasons such as the dose or virulence of organisms, the route of innoculation or criteria for measuring the severity of infection.

Results with the two pairs of mouse strains which shared either the b or k allele at the H-2 K and A loci suggested that resistance to the late expansion of tuberculous infection and granulomatous infiltration of lungs was largely associated with the absence of expression of the E genes and the presence of the D^b allele. Either of these genes could influence the protective or pathogenic host response by mechanisms which have been implicated in previous immunogenetic studies of various murine infections. The most pertinent analogy could be drawn with the reports that resistance to intestinal nematode infections (*Trichinella* and *Namatospiroides*) has been associated with the lack of E-gene expression in B10 mice of four different H-2 haplotypes.[17] This was interpreted in terms of suppression of H-2A-restricted protective immunity by H-2E-restricted T cells and sustained by the finding of the susceptible phenotype in F_1 hybrids.[18] Wassom and colleagues proposed a regulatory (suppressive) role of E-locus-restricted T-cell responses with specificity either to antigenic epitopes[19] or to autologous epitopes on lymphocytes or accessory cells.[20] This concept is also supported by the finding that injection of anti-IE monoclonal antibody can enhance parasite clearance from organs of *Leishmania donovani*-infected mice.[21] In accordance with the present results on chronic tuberculous lung infection, both the therapeutic effect of anti-IE treatment and the H-2E-restricted association occur only in the late phase of both parasite and nematode infections. The H-2E-restriction of T cells in protracted lung or gut localized immune reactions could involve macrophages which have distinct enzyme or bactericidal activities.[22,23] Selective E-gene expression on lung accessory cells could influence the predilective localization and extravasation of lymphocytes into lung parenchyma,[24] or could regulate the suppressive activity of lung macrophages on T-cell responses.[25] The complex cellular events may also result in reciprocal outcome for some pathogens such as *Salmonella typhimurium*, where E^- strains were found susceptible and E^+ strains resistant in the late phase of infection.[26]

As an alternative interpretation, it is possible to consider the role of the $H\text{-}2^b$ gene which associated with restrained lung infection when compared with either D^d or D^k strain. This interpretation would be in accord with the generally postulated protective role of class I-restricted cytotoxic CD8 T cells[27] and with the reported association of $H\text{-}2D^b$ with protection to 3-week levels of infection with *S. typhimurium*.[28] However, the number of proteins or fragments of proteins derived from mycobacteria inside the phagolysosome which are able to gain access to the cytoplasm and hence associate with class I molecules may be more limited than those which can associate with class II molecules.

Although many genes apart from the classical class I and class II genes map to the H-2 region, these tend to be less polymorphic, and therefore less likely to underlie the differences in control of *M. tuberculosis* observed between different H-2 haplotypes. Nevertheless an increasing number of genes which could be important in controlling bacterial infections have been mapped to the major histocompatibility complex (MHC) region, e.g. tumour necrosis factor-alpha (TNF-α) peptide transporter proteins, the multi-drug resistance family of transmembrane transporters and the heat-shock protein HSP70.[29–32] It is possible that differences in the regulation of expression of genes encoding such molecules may occur between different H-2 haplotypes.

Note added in proof

In a recent experiment, lung CFU counts in (B10 × B10.BR)F_1 mice were corresponding or slightly above those found in the resistant B10 strain phenotype. This finding is at variance with the susceptible F_1 phenotype following nematode infections.[18]

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