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EDITORIAL REVIEW

Susceptibility to tuberculosis – the importance of the pathogen as well as the host

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Tuberculosis remains one of the largest causes of death from an infectious agent, with 8 million new cases and 2 million deaths per annum [1]. It is also estimated that one third of the world's population is latently infected with *Mycobacterium tuberculosis*. The vast majority of these individuals do not develop disease. The improved control of this pathogen will depend on a better understanding of the many environmental and host genetic factors that determine the progression from latent infection to disease. The most important environmental factor, on a global scale, is HIV infection. Co-infection with HIV increases the risk of developing disease from 10% in a lifetime to a 10% annual risk [2]. There is evidence from twin studies of a genetic component to susceptibility to tuberculosis [3] but no single major tuberculosis susceptibility gene has yet been identified. However, studies have identified polymorphisms within a few genes such as the NRAMP1 gene and vitamin D receptor that are associated with smear positive pulmonary tuberculosis [4,5], and these genes may contribute to the genetic susceptibility to tuberculosis found in twin studies.

Whilst environmental and host genetic factors are important in determining progression to disease, factors within the pathogen may also play a role. A greater understanding of the complex host–pathogen interaction has evolved from the study of different laboratory and clinical isolates of *M. tuberculosis*. For many years, *M. tuberculosis* was considered to be extremely highly conserved with a high degree of sequence homology and lack of antigenic diversity [6,7]. Whilst this is true, the development of DNA fingerprinting techniques have allowed the demonstration of areas of the *M. tuberculosis* genome where a high degree of DNA polymorphism is associated with repetitive DNA sequences and insertion elements [8]. DNA typing of one of these areas of sequence insertion, IS6110, has been widely used to differentiate different clinical isolates in epidemiological studies. Differences in sequence between different clinical isolates can be associated with differences in either infectivity or virulence. The investigation of an outbreak of a newly identified, genetically distinct strain of *M. tuberculosis* (now known as strain CDC1551) [9] revealed that this strain had an unusually high rate of transmission in humans and was significantly more virulent in animal models than other clinical isolates. Detailed immunological studies of this strain have shown that it induced higher levels of TNF-α, IL-10, IL-6 and IFN-γ than other strains in the lungs of infected mice and when grown in human monocytes [10]. The completion of the genome sequence of the standard laboratory strain of *M. tuberculosis*, H37Rv [11] and the subsequent sequencing of the CDC1551 strain [12] has allowed a direct genomic comparison between these strains to be made. Single nucleotide polymorphisms were found in many different genes. The clinical and epidemiological differences in this strain have therefore now been linked with both immunological and genetic differences.

The investigation of other clinical isolates has provided further evidence of the importance of the nature of the host immune response to the pathogen. In the study by Manca *et al*. [13], the clinical isolate, HN878, was found to be hypervirulent and mice infected with this strain failed to induce a Th1-type immune response, with lower levels of IFN-γ and TNF-α in the lungs.

The association between pathogen sequence diversity and variation in host immune response demonstrates the importance of investigating genetic differences between strains of *M. tuberculosis*. The development of a Th1-type immune response is essential for protective immunity against *M. tuberculosis*. *M. tuberculosis* is an intracellular pathogen residing primarily in macrophages. As such, the activation of macrophages, primarily by IFN-γ, is central to the protective immune response in mice and humans [14]. If sequence diversity results in differing ability to induce a host Th1-type immune response, then this may explain different patterns of disease.

In this issue of *Clinical and Experimental Immunology*, Lopez *et al*. [15] take this work one step further. They have used the mouse model of pulmonary tuberculosis to investigate the role of genetic diversity in determining pathogenicity and immunogenicity. They have taken 12 distinct strains of *M. tuberculosis*, defined on the basis of IS6110 RFLP patterns and representing 4 major genotype families found throughout the world today. They investigated the immune response, survival after challenge and histopathological changes in mice infected with these different strains and compared these with infection by a standard laboratory strain, H37Rv. They also investigated the protective efficacy of BCG against these different strains. They found marked differences in the patterns of cytokine induction and development of immunopathology between the different strains. Mice infected with the Beijing genotype had a significantly higher bacillary load

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and mortality rate at 4 weeks postinfection. Mice infected with the Canetti strain had a lower bacillary load and significantly smaller areas of pneumonia when compared with the Beijing type. The differences between the other strains were less marked and showed intermediate rates of survival. The protective efficacy of BCG against the different strains of *M. tuberculosis* was found to vary and BCG was least protective against the Beijing strain.

The patterns of cytokine secretion are intriguing. The secretion of IFN-γ is generally taken to be the best identified correlate of protection [16], however, the different mortality rates between strains in this study cannot be explained by the pattern of IFN-γ secretion found. The Beijing and Canetti strains induce similar levels of IFN-γ late in infection despite these 2 strains inducing very different mortality rates. It may be that the early secretion of IFN-γ in mice infected with Canetti strain is important. Infection with Canetti strain induces a constant level of TNF-α, in contrast to the Beijing strain which induces TNF-α only at the early stages. The secretion of iNOS follows the pattern of TNF-α secretion. The relative contributions of these and other factors to the mortality associated with different strains requires further clarification.

The findings of this study are important and pertinent to TB research today. The Beijing genotype is the predominant strain of *M. tuberculosis* in several distinct geographical areas, presumably because of a selective advantage of this strain over other strains [17]. The failure to induce a protective immune response and the impaired protective efficacy of BCG provide an immunological basis for this selective advantage.

The evaluation of protective efficacy of new candidate TB vaccines typically involves the use of a single laboratory strain of *M. tuberculosis*, H37Rv in animal challenge studies. These findings suggest that it may be important for new candidate vaccines to be evaluated using a wider range of laboratory and clinical isolates.

The application of microarrays should help us to characterize the genetic differences between strains in more detail. The exact sequence changes causing the strain differences remain undefined. It could be the insertion sequences that are responsible but it is perhaps more likely to be particular SNPs. Further animal studies using different clinical and laboratory isolates are necessary to evaluate how these genetic differences translate into functional differences in infectivity, virulence and cytokine induction. The identification of genes responsible for virulence will allow the development of attenuated strains of *M. tuberculosis* and potential vaccine candidates based on such auxotropic strains. A greater understanding of the different patterns of cytokine secretion between different strains and how this translates into virulence will aid the identification of the elusive correlates of protection that are needed for the clinical evaluation of the most promising vaccine candidates. These studies will help bring the ultimate goal of improved TB control and eventual eradication one step closer.

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