FROM BCG/LSH/ITY TO NRAMP1: THREE DECADES OF SEARCH AND RESEARCH

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Gene identification in the pre-genomic era was often arduous, and the mouse Nramp1 gene (natural resistance-associated macrophage protein 1; formerly the Bcg/Lsh/Ity locus) was no exception. Yet, the three decades of gene search and research which have encompassed Nramp1 can be viewed as a journey that produced not one, but many discoveries. In this review, we provide a brief history of the Nramp1 gene, its discovery and the ongoing efforts to characterize the role of the mouse and human NRAMP1 genes in susceptibility to mycobacterial infections.

Early Studies on the Bcg/Lsh/Ity Locus

By 1974 the groundwork was already laid for the existence of a single gene in mice, with resistant and susceptible alleles that controlled the growth of intracellular parasites (27th Forum in Immunology, 1989). The group of Bradley had reported the different growth of Leishmania donovani among inbred strains of mice, terming the locus Lsh. The group of Plant and Glynn observed a similar phenomenon in Salmonella typhimurium infection, and termed their locus Ity. Our group entered the scene in 1981 with the report that inbred mice strains segregated as resistant or susceptible to infection with small doses of Mycobacterium bovis, terming the locus Bcg. All groups quickly realized a common gene was at work as the Lsh, Ity, and Bcg genes were independently mapped to the same location on chromosome 1 (Mock et al., 1990; Skamene, 1994). In 1982, two back-to-back papers in Nature confirmed that the resistance to the three pathogens was controlled by the same locus (Plant et al., 1982; Skamene et al., 1982). The papers were notable not only for the extensive linkage analysis to different infections by progeny testing, but also for the predictions that were ventured. It was proposed that a single gene regulated resistance to the three taxonomically distinct pathogens and that the gene would likely function in the intracellular milieu of the macrophage by affecting a shared biochemical or nutritional growth requirement. Importantly, it was also surmised that the relatively small number of susceptible inbred strains implied that the susceptible allele was a recent mutation and not a variant of a polymorphic gene. Finally, it was proposed that a human homolog of Bcg would regulate resistance to tuberculosis and leprosy. Time has shown the remarkable accuracy of those early predictions, however, in 1982, the sole task was to find out whether Bcg/Lsh/Ity was one or three closely linked genes. Yet, without knowledge of a protein product or gene function, this undertaking would entail positional cloning, or “reverse genetics”, as it was then known, a process by which no mouse gene had ever been identified (Gros and Malo, 1989).

From Bcg/Lsh/Ity to Nramp1

The subsequent 10 years of research on Bcg/Lsh/Ity involved a significant risk for the cloning laboratories. On the one hand, although they could count on the positional cloning strategy to ensure eventual arrival at the gene, the project was extremely work intensive, especially in a time when new techniques of recombinant DNA technology were just being implemented. In addition, there was always the possibility of another laboratory arriving at the gene through a protein or functional analysis. In fact, several groups, including our own, were pursuing the Bcg/Lsh/Ity gene by systematically comparing aspects of macrophage function in Bcg congenic mice, such as analysis of surface receptors, signaling proteins and bactericidal mechanisms (27th Forum in Immunology, 1989). In addition, attempts were made to identify the putative macrophage Bcg product by raising antibodies or through two-dimensional gel analysis. Although these studies did accumulate very valuable information on macrophage function, they did not lead to the gene. Rather, the Bcg/Lsh/Ity gene appeared to be an elusive director of “pleiotropic effects of macrophage activation”, which appeared downstream of the actual gene function. These pleiotropic effects were manifested as up-regulation of numerous functions, including class II antigen expression, nitric oxide, protein kinase C, and antigen presentation (Blackwell et al., 1991; Buschman et al., 1995; Zwilling and Hillburger, 1994). Along with these pleiotropic effects, there were several suggestions for the Bcg gene product including a DNA-binding protein like NF-κB, an autocrine interleukin, a protein kinase C transporter, and a cytoskeletal protein. Meanwhile, the cloning group, following an intensive period of narrowing down the Bcg region by genetic and physical mapping, had a breakthrough in 1992 using the technique of exon amplification reported the previous year. This method was used to detect competent exons within cloned genomic fragments, and resulted in the discovery of Nramp1, the first mouse gene obtained by positional cloning (Vidal et al., 1993). Within the next year, the 5’ promoter region of Nramp1 was described (Barton et al., 1994), followed quickly by the cloning of human NRAMP1 and a second mouse Nramp2 gene (Cellier et al., 1994; Gruenheid et al., 1995). The next year saw the creation of Nramp1 knockouts and transgenics, and the long sought antibody against the Nramp1 protein was finally produced (Gruenheid and Gros, 2000).

The Role of Nramp1 in Mycobacterial Disease

By 1996, all the predictions from 1982 were verified (Skamene et al., 1998). First, the susceptible allele did turn out to have a recent, single amino acid mutation in the fourth transmembrane domain that resulted in absence of the protein. Second, the Bcg/Lsh/Ity locus was confirmed to be one gene, by analysis of infection in knockouts and transgenics. Third, the Nramp1 protein was revealed to be an intracellular macrophage protein, situated in the phagosomal membrane and functioning as a divalent cation transporter. Although the precise mechanism of Nramp1 function is still to be worked out, it appears...
that removal of divalent cations from the phagosome could both restrain pathogen growth and result in the “pleiotropic effects” of macrophage activation. Finally, the existence of a human homolog controlling resistance to tuberculosis and leprosy was proven true, but with the caveat that human NRAMP1 is a modulator of disease susceptibility, since only NRAMP1 variants have been shown to account for partial susceptibility to disease. Surprisingly, another venerable prediction that still remained to be tested, namely, that Nramp1 regulates resistance to Mycobacterium tuberculosis, has proven not to be true (Medina and North, 1996). These studies have resulted in an unusual situation where it is human NRAMP1, and not mouse Nramp1, that has been linked with tuberculosis. It is this current paradox that we would like to put in more perspective by discussing issues relevant to mouse and human mycobacterial disease.

Relevance of Nramp1-Knockout Studies to Tuberculosis

The recent gene knockout studies of Nramp1, on the 129/J background, have clearly shown that Nramp1 does not affect resistance to the H37Rv (virulent human isolate) strain of M. tuberculosis, with Nramp1-resistant and -susceptible mice displaying an equivalent susceptibility to M. tuberculosis, in all organs examined (North et al., 1999a). These results fit with earlier studies showing that the pattern of susceptibility and resistance to M. tuberculosis did not follow the allelic expression of the Bcg gene in inbred or Bcg-congenic mice (Medina and North, 1996, 1998). There are some experiments needed before Nramp1 can be confirmed to have no role in resistance to M. tuberculosis. Specifically, the global effect of Nramp1 in mouse tuberculosis should be examined in a series of Nramp1 knockouts on several genetic backgrounds, with different strains of M. tuberculosis. Assuming that Nramp1 has no role in resistance to M. tuberculosis, the fact that the human NRAMP1 gene is associated with susceptibility to tuberculosis means that there is either a fundamental difference between mouse and human Nramp1 (an unlikely event considering the high degree of homology) or between the mouse model and human tuberculosis (much more likely). Thus, a re-examination of the mouse model of tuberculosis is warranted. First, since it has been firmly established in humans as well as in mice that susceptibility to M. tuberculosis is a complex, multigenic trait, multigenic control and epistatic interactions should be examined through QTL and recombinate congenic strain analysis, rather than knockout, approaches (Kramnik et al., 1998; Lavebratt et al., 1999). Second, since both bacterial burden and median survival time in mice are problematic indicators of M. tuberculosis resistance, the use of other parameters such as infection-triggered cachexia or lung pathology may be more useful models for human disease (Nikonenko et al., 1996; Lavebratt et al., 1999; North et al., 1999b). Finally, because M. tuberculosis is not a natural pathogen of inbred mice, the mouse model employing M. tuberculosis may be of limited use compared with human tuberculosis, where M. tuberculosis is a natural pathogen. The use of other animal species in the analysis of genetic resistance to M. tuberculosis may represent better models. On the other hand, the use of mycobacterial strains, which have been a natural force of selective pressure in the mouse, could perhaps identify genetic loci in wild-derived mouse strains.

Diverse Tissue Expression of Nramp1/NRAMP1

Another point to consider regarding the diverse results of mouse and human NRAMP1 with respect to tuberculosis is that a different pattern of Nramp1/NRAMP1 tissue expression exists in mice versus humans. In Northern blot studies to detect mRNA transcript expression, mouse Nramp1 was strongly expressed in the spleen and liver, with almost no detectable expression in the lung (Vidal et al., 1993; Govoni et al., 1995, 1999). At the cellular level, Nramp1 mRNA is found in the myeloid cells of the bone marrow, professional phagocytes and can be up-regulated upon exposure to cytokines. Looking back to past results, these data fit very well with observations that the phenotypic expression of the Bcg/Lsb/lty gene is most strong in the spleen and liver in regulating resistance to the pathogens M. bovis, Mycobacterium lepraevarium, M. avium, S. typhimurium, and L. donovani; all pathogens that do not parasitize lung tissue. In contrast, M. tuberculosis has a defined tropism for the lung, and all fatal pathology is associated exclusively with the lung. In humans, NRAMP1 expression was detected more strongly in the lung compared with the spleen and liver (Cellier et al., 1994, 1997). In addition, NRAMP1 expression was found most strongly in PMNs rather than in the fixed tissue macrophages of professional phagocytes found in the mouse. The reason for the different tissue expression of the Nramp1 gene in mouse versus humans is unknown, but it is possible that the strong expression of human NRAMP1 in the lung and PMNs has actually been driven by the evolutionary pressure of M. tuberculosis infection.

NRAMP1 in Humans—A Determinant of Acquired Immunity?

Strong linkage of tuberculosis to NRAMP1 has been detected in a tuberculosis outbreak in a community of aboriginal Canadians (Greenwood et al., 2000) and also in a Gambian population in West Africa (Bellamy et al., 1998). For leprosy, a role of NRAMP1 in susceptibility to leprosy was obtained in a large familial study in South Vietnam (Abel et al., 1998). Other studies have failed to show linkage of tuberculosis and leprosy to NRAMP1 (Roger et al., 1997; Shaw et al., 1997). The overall picture is that the influence of NRAMP1 over susceptibility to tuberculosis and leprosy is genetically heterogeneous and dependent upon the immunization status of the population.

In this context, another study of NRAMP1 in leprosy has raised an important issue. In this study in Vietnam, significant linkage was observed between NRAMP1 and Mitsuda reaction, an in vivo diagnostic test for lepromatous leprosy that measures the specific immune response against lepromin (Alcais et al., 2000). These results imply that NRAMP1 plays a regulatory role for the development of acquired antimycobacterial immune responses. Thus, although it is firmly accepted that mouse Nramp1 is a determinant of natural resistance to infection, and not acquired immunity, the human studies do not unequivocally show that this is the case for NRAMP1. It remains possible that, in humans, NRAMP1 could represent an immune response gene.

Summary and Future Predictions

In the mouse, the Nramp1 gene is a determinant of natural resistance to several mycobacterial pathogens but not to the human lung pathogen H37Rv M. tuberculosis. In humans, NRAMP1 has been shown to modulate susceptibility to M. tuberculosis and Mycobacterium leprae, depending on the epidemiologic infection setting and the ethnic background of the population. Thus, natural selection by different infectious agents in humans and mice may have caused the highly homologous Nramp1 gene to diverge in terms of tissue expression, gene variants, and gene interaction. These divergences have also called into question the validity of the mouse M. tuberculosis model. However, the studies regarding the function of Nramp1 in mice are still highly relevant, because the structural similarity of mouse and human NRAMP1 implies that the basic function will be conserved. In fact, such functional studies will have wide relevance,
since the Nrap1 genes have been highly conserved throughout evolution, from bacteria to mammals.

We have observed over the history of the Bcg/Lsh/Ity gene the crossing over of scientific disciplines, which has fostered much opportunity. Recently, the field of genome sequencing of bacteria has merged with the field of population epidemiology to produce “molecular epidemiology”, which has challenged decades of dogma and provided crucial information on how tuberculosis can spread and why the BCG vaccine has failed (Behr et al., 1999; Behr and Small, 1999; Kato-Maeda and Small, 2000). We envision the next step for host resistance to infectious diseases will be a merging of mouse infection models employing genomically sequenced macrophage. This approach is likely to reveal links between specific bacterial virulence genes and host resistance genes. These links may lead to therapies based on specific host-pathogen mechanisms, which could represent a way out of antibiotic treatment of tuberculosis.

References


