Special Session : Animal Models

SPL-1

Immunological Perspective of Leprosy Using Transgenic and Knockout Mouse Models

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Leprosy is classified along a spectrum based on clinical and histopathological criteria. Toward the tuberculoid end of the spectrum, leprosy lesions feature strong cell mediated immunity and contain few acid fast bacilli, while at the lepromatous end of the spectrum cell mediated immunity is poorly developed and lesions contain numerous acid fast bacilli. Immunological mechanisms are believed to relegate a patient to a particular position on the spectrum and modulate upgrading and downgrading responses in the borderline areas. In this regard, associations have been made between tuberculoid leprosy and Th1-type responses and lepromatous leprosy and Th2-type responses; however, whether the cytokines present are the cause or an effect of the disease classification is poorly understood. The mouse model has contributed valuable information to our understanding of leprosy. Upon infection with *Mycobacterium leprae*, conventional, immunocompetent mice permit only limited growth of the bacilli and the histopathological changes are minor, both attributes of indeterminate leprosy. In contrast, athymic *nu/nu* mice show characteristics of lepromatous disease in that they allow tremendous growth of *M. leprae* and the infected foot pad tissue consists of heavily infected foamy macrophages. The development of genetically engineered mice, particularly those with defects in immune pathways important in host defense, has allowed investigation of discreet steps in the immune response, as well as their interactions, over the course of this chronic infection. The strains that we are studying represent impediments to both innate and acquired immunity and are considered important in the host response to intracellular pathogens at the level of both macrophage and T cell effector functions or in immunoregulation in the localized microenvironment of the granuloma (e.g., NOS2$^{-/-}$, gp91$^{phox-/-}$, TNF$^{-/-}$, TNFR1$^{-/-}$, IL-12/23p40$^{-/-}$, IFNg$^{-/-}$, IL-10$^{-/-}$, CD4$^{-/-}$, CD8$^{-/-}$, *nu/nu*). In addition, we are examining strains which carry mutations in genes which have recently been identified as major risk factors for leprosy (i.e. LTa$^{-/-}$ and PARK2$^{-/-}$). Specifically, we are evaluating the disease which develops in various knockout mice upon foot pad infection with viable *M. leprae* using both the Shepard growth model and a modified Lepromin Test model. In addition, we are modifying disease in the appropriate knockout strains by treatment with immunomodifiers to create an additional knockout, and modifying established disease in the appropriate knockout strains by restoring the deleted function. We are monitoring bacterial growth, granuloma formation, cytokine and chemokine responses, and cellular infiltration, distribution and evolution. We are also using cells isolated from foot pad granulomas to construct in vitro granulomas. These studies have determined that certain knockout strains yield models of infection which can be classified along the leprosy spectrum. Other knockout strains, while not easily classified, exhibit thought-provoking phenotypes. We have found that administration of immunomodulators can induce a change in the disease presented in certain knockout strains, demonstrating that the *M. leprae*-induced mouse foot pad granuloma is a dynamic entity and has potential for determining the mechanisms underlying the instability of borderline leprosy. Furthermore, we have found that the cell profiles from the foot pad granuloma are quite distinct from the more easily assessable draining lymph nodes. Finally, cells isolated directly from the foot pad granuloma respond to *M. leprae* antigens in vitro and may yield insights into and support selection of specific antigens for use as diagnostic tools and vaccine candidates.

SPL-2 The Armadillo : A model Exhibiting the Full-Spectrum of Leprosy

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The nine-banded armadillo, *Dasypus novemcinctus*, is uniquely susceptible to infection with *Mycobacterium leprae*, and has developed to be the host of choice for *in vivo* propagation of leprosy bacilli. The bulk quantities of *M. leprae* made available through armadillos has been a boon to leprosy research, but, like man, the specific factors that underlie their unique susceptibility to infection remain unclear. Though the majority of armadillos manifest lepromatous-type responses, some armadillos are wholly resistant to infection and others manifest borderline or tuberculoid-like responses when exposed to *M. leprae*. This *full* spectrum of immunological activity towards *M. leprae* recommends them as model hosts for translational studies to develop new diagnostic assays and anti-leprosy vaccines. Armadillos closely recapitulate leprosy as seen in man and they exhibit many of the same clinical problems of the infection. Paramount among these is extensive involvement of the peripheral nerves which can contribute to chronic ulcers in the extremities and other issues associated with insensitive limbs. Nerve conduction velocity testing is evolving as a means to index leprosy associated impairment in these animals, and armadillos are the most abundant source of leprotic neural fibers. Nine-banded armadillos in parts of the southern U.S. also are known to harbor a natural infection with *M. leprae*. It appears to have evolved by natural means and the disease is most common among armadillos in low-land habitats. Armadillos can support intense transmission of *M. leprae* in the wild, and incidence densities as high as 3.5 cases per 1000 animal days have been measured. More than 100,000 infected armadillos are estimated to range in Louisiana and Texas. However, the risks this sylvan infection presents to man and the total geographical extents of its range remain uncertain. Armadillos do not require specialized housing but can be incorporated into many existing animal facilities. Their long life span, cool body temperature and unusual reproductive cycle can recommend them for a variety of studies. The main factors limiting their use as laboratory models has been a paucity of specific biochemical or immunological reagents, and our inability to breed the animals in captivity. Recently, the Human Genome Consortium completed a 6X coverage sequence of the armadillo genome and has begun to annotate that sequence data in comparison to humans. This new genomic data is helping to make available a vast array of biochemical and immunological reagents and will likely facilitate more extensive use of armadillos in other research studies. Though 35 years has passed since armadillos were shown to be susceptible to *M. leprae* their importance as model hosts in the study of leprosy may be only just beginning.

SPL-3 Study of Pathomechanism/s of Nerve Damage in Leprosy Using Mouse Model

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While screening drugs for antileprosy activity and testing for bacterial viability are the two most important practical applications of mouse model, in depth studies investigating the peripheral nerves in the experimental model are very few (Johnstone 1987). We summarize our contribution of past 25 years towards understanding of evolution and mechanism/s of nerve damage where the mouse model was used as an adjunct to human studies. The findings vindicate its role in deciphering of the sequence of events and mechanisms in leprous neuropathy. Structural and electrophysiological (NCV) changes were noted as early as 4 months in the sciatic nerves of Swiss white (S/W) mice injected in the foot pad with freshly harvested human derived *M.leprae* (5000 *M.leprae* / foot pad). Primary involvement of '.C' fibres followed by atrophic changes in the axons, paranodal demyelination and segmental demyelination noted were similar to that seen in early human leprous nerve lesions (Jacobs et al 1987,Shetty et al 1988).In vitro NCV recording of the excised mouse nerves reveled absence of 'C' fibres potentials at 3 month and changes in A a fibre potentials at 6th month demonstrating that the functional changes precede structural changes (Vidyasagar et al 1981). Blood nerve and perineurial barriers were intact up till 18 month post infection despite significant loss of fibers. However integral bacilli, its antigens and granulomatous reaction were conspicuously absent in the involved mouse nerves inoculated in the footpad with *M.leprae* (Shetty et al 1980). When freshly harvested *M leprae* were directly injected in to the sciatic nerves of normal and immuno suppressed mice, tuberculoid and lepromatous type of neural granulomas were noted at 2 and 4 weeks time respectively. Two striking findings were a) lesion remained localized and b) *M.leprae* failed to enter the Schwann cells (Shetty and Antia 1999, 2002). **Model for characterization of *M.leprae:*** In order to determine whether *M.leprae* alone produced the typical change in the sciatic nerve of foot pad inoculated mice, a comparative study was undertaken using various other mycobacteria. Typical changes described above were seen only with *M.leprae* (Kamala et al 1984). **Effect of immunomodulation** using cyclosporine A, Thy 1.2, and T-200x5R model, on nerve lesion were also studied. Highlights of the findings were1) The significance of non-T cell mediated antibacterial mechanisms 2) Metabolic interaction between *M.leprae* and host cell and 3) Dissociation between *M.leprae* growth in the foot pad and nerve damage (Shetty et al 1995). Recent and on going studies are aimed at bridging the gap between cause to effect relationship. Findings show that *M.leprae* mediated dephosphorylation lead to collapse of axon (atrophy) that result in' paranodal and nodal demyelination (Shetty et al.1999 Save et al 2004).