Review Article

LEISHMANIASIS: BIOLOGICAL UNDERSTANDING AND BEYOND

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SUMMARY: An attempt has been made to briefly review the literature regarding current molecular understanding of host parasite relationship in Leishmaniasis. Sequencing of genomes for both human and important microbes including *Leishmania major* are in progress. The genetic information from both human and parasite have led towards molecular understanding of the interaction between parasite virulence factors and the host response factors. Gene codes for natural resistance associated macrophage protein 1 (NRAMP1), which controls the susceptibility to *Leishmania donovani, Salmonella typhimurium* and *Mycobacterium bovis* has been cloned in both mice and human. Research into the host genetics of Leishmaniasis has revealed the fundamental immunological mechanisms determining outcome of infection. It has been shown very conclusively that Tumour Necrosis Factor Alpha (TNF α) MHC and T helper cells (Th) all are associated to determine the susceptibility or resistance to leishmanial infection. Biological insights regarding parasite virulence genes identified as part of the leishmania genome project may be specifically targeted for vaccines or designing drugs for new treatments.

KEY WORDS: Leishmaniasis

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INTRODUCTION

Leishmaniasis are caused by haemoflagellates protozoan, which are exclusively transmitted by the bite of a tiny 2 to 3 mm long female sand fly of the genus Phlebotomus in the Old World and Lutzomyia in the New World. The disease is geographically and ecologically widespread, occurring in tropical and subtropical regions on all continents except Australia and endemic in 88 countries. Worldwide, two million new cases of leishmaniasis occur each year, and possibly a 10th of the world's population is at risk of infection[1,2]. Although the disease is highly endemic in certain geographical areas like North Africa, the Middle East, parts of Europe, central & South America and many parts of the Indian subcontinent but epidemics are well recognized. It is prevailing at the disastrous level in southern Sudan where more than 10% of the population has died from visceral leishmaniasis over the past five years. The incidence of Leishmaniasis is increasing, with many endemic areas reporting a 500% increase over the past seven years[3].

Clinically, the diseases are mainly classified as visceral (VL), cutaneous (CL) mucocutaneous (MCL), diffuse cutaneous (DCL) and post-kala-azar dermal (PKDL) leishmaniasis[4]. Among these clinical cases, VL is the most

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serious forms and of all VL cases in the world, 90% cases occur in Bangladesh, India, Sudan and Brazil[5].

With the global spread of HIV, visceral leishmaniasis has become increasingly prevalent and unusual presentations often occur. Leishmania / HIV co-infection has emerged as a result of the increasing overlap between VL and AIDS. In the Mediterranean endemic areas, approximately 50% of all VL cases in adults are associated with HIV infection[6]. Reactivation of asymptomatic or previously 'healed' leishmania infections is common with the onset of AIDS and Leishmania species that normally cause only cutaneous disease may present with visceral leishmaniasis in a patient[7]. Importantly, co-infections of Leishmania and HIV are often resistant to treatment and consequently accelerate the progress of AIDS[8].

Clinical presentations or outcome of infection caused by Leishmania are determined by interactions between the host and parasite, which are governed by their genomes. It is therefore very exciting to note that both host and parasite genomes have been targeted for sequence analysis. The genetic information from both human and parasite and the emergence of new tools such as microarray technologies will allow us to gain an understanding of the interaction between parasites virulence factors and host response factors. Molecular knowledge thus obtained will facilitate targeting of new treatments.

Genetic & Immunological understanding

Leishmaniasis constitutes a diverse collection of human diseases ranging from a disfiguring cutaneous and mucocutaneous lesion that can cause widespread destruction of mucous membranes to visceral disease affecting the haemopoetic organs. Molecular genetic analysis has revealed at least 21 different Leishmania species belonging to either subgenus Leishmania or Viannia. For epidemiological and therapeutic purposes, identification of Leishmania species is very important. With the advent of continuous researches many steps in the molecular pathogenesis of leishmaniasis are now well understood. During its time in a sandfly gut as a motile flagellated promastigote stage, a biochemical modification of the parasite's glycolipid coat occurs. This important transformation protects the parasites from rapid lysis via the mammalian complement system when it enters a host. The leishmania parasite is an intracellular pathogen of the immune system. Once the parasite has gained access to the mammalian host through a sandfly bite, it is taken up via receptor-mediated phagocytosis by resident skin dendritic cells or macrophages and the parasites differentiate into the amastigote form[9]. In an elegant example of molecular mimicry, the organism uses the host complement receptors to gain access to the hostile environment of the phagolysosome. Despite a low pH of 4.5 to 5.0 and activated proteinases in the phagolysosome, it thrives. Later on activated macrophages and helper T lymphocytes are recruited to the site of infection through events of cell-mediated immune response. The pathology resulting from infection with Leishmania substantially depends on host genetic factors. Several of these factors have recently been identified using genetic approaches in both mice and humans. Analysis of mouse chromosome 1 revealed that susceptibility to Leishmania donovani, Salmonella typhimurium and Mycobacterium bovis was controlled by the same gene located on this chromosome[10]. This gene which codes for natural resistance associated macrophage protein 1 (NRAMP1) was subsequently cloned from mice and then human[11,12].

Interestingly although the gene may not play a major role in human leishmaniasis[13], a recent West African study showed that humans with specific alleles of the NRAMP1 gene were significantly more likely to have tuberculosis[14]. While no association has been seen in humans between allelic forms of NRAMP1 and leishmaniasis, an understanding of its action may still help explain the peculiar ability the parasite has of surviving the harsh environment of the phagolysosome.

Molecular pathogenesis of mucosal leishmaniasis, which might cause grossly disfiguring complication of a previously healed cutaneous infection in only a small percentage of individuals, still remains a puzzle. Again host genetic factors have now been implicated in the pathogenesis of mucosal disease. In a study of a Venezuelan population it was found that particular alleles encoding the cytokines *'tumour necrosis factor'* Alpha & Beta (TNF α and TNF β) were associated with significantly increased relative risks (3.5 and 7.5 respectively) of mucosal disease[15]. TNF α was over expressed from the allele associated with disease, an observation that fits well with other reports of high concentration of TNF α found in patients with leishmaniasis. This cytokine releasing gene was further implicated by a mouse study where one of the two receptors of TNF α was ablated by means of gene targeting. The mice lacking the receptor could not heal cutaneous ulcers despite being able to control parasite replication[16]. Depending on these findings, TNF α is being considered as a candidate for pharmacological intervention.

For a disease in which cell mediated immunity plays a central role, it is not surprising that the major histocompatibility complex (MHC) is intimately involved. In mice it was shown that different major histocompatibility complexes were associated with different susceptibilities to visceral leishmaniasis[17]. Role of MHC in cutaneous leishmaniasis has been described in humans[18] and endorsed by a genetic linkage study in mice[19]. These data add to the wealth of evidence supporting a role for the MHC in resistance to a variety of infectious diseases including leprosy, schistosomiasis, malaria and hepatitis B and in the progression of HIV infection to AIDS[20,21]. The mouse model of infection with Leishmania major helped to explore the cellular basis of this phenomenon. Heinzel et al[22] correlated that the outcome of infection is determined by the nature and magnitude of T cells and cytokine response early in infection. In infected inbred mice, production of IFN-γ by Th1 and natural killer (NK) cells mediate resistance, while, expansion of IL-4 producing Th2 cells confers susceptibility[23]. The classification of T helper cells into Th1 or Th2 was based on the distinct cocktails of cytokines the cells secrete[24]. Environment produced by these different cytokines of two subsets of T helper cells in turn recruits and activates different immune effector cells[25]. Moreover, once established, these responses become mutually exclusive to a large extent. In this way the type of T helper cell response determines susceptibility or resistance to leishmanial infection.

Although there are currently no data from humans regarding susceptibility to cutaneous leishmaniasis, studies in mice suggest that at least three interacting genes may be responsible for such susceptibility[19,26]. Finding such genes might provide a mechanism for switching from deleterious to a protective T helper cell response.

The Future

Genome sequencing of both human and L. major are in progress with the speculation to be completed in the next few years. The Pathogen Sequencing Unit at the Sanger Institute has played a major role in the genome sequencing of L. major, which has an estimated size of 33.6 Mb with a karyotype of 36 chromosomes[27]. Information to be obtained from sequencing of genomes will allow the study of disease pathogenesis from two perspectives. Firstly, the human genome sequence will accelerate the identification of the genes governing host susceptibility and resistance to many microbial diseases including leishmaniasis. Secondly, the leishmania genes that determine virulence will be identified as well. Through these findings, question such as how the parasite survives the hostile phagolysosomal environment of macrophage and which parasite and host factors determine the type of T helper cell response will be answered. More importantly, these biological insights will yield targets for new treatments. Knowledge of all leishmania genes will greatly increase the options for appropriate vaccine design in near

future. The ensuing bright future is not limited to leishmaniasis research only, in fact, genome projects are in progress for many major infectious diseases including tuberculosis, malaria, schistosomiasis, filariasis, trypanosomiasis and for many pathogenic bacteria[27].

CONCLUSION

Biological understanding of leishmaniasis can be used for designing therapeutic drugs in several ways. Genes responsible for modifying host susceptibility will be the prime target for therapy and protein encoded by such gene can be used directly. Examples of similar product are already in clinical practice include interferon alfa for treating hepatitis C and granulocyte colony-stimulating factor for cancer chemotherapy. Alternatively, it is possible to produce monoclonal antibodies to enhance or block a gene expressed protein product, such as monoclonal antibodies to tumour necrosis factor Alpha (TNF α) used in Crohn's disease.

Greater understanding of the genetic basis for individual susceptibility to leishmaniasis may allow individuals at high risk to be targeted for priority vaccination and early treatment strategies. Through human genome project, genetically susceptible persons to leishmaniasis will be identified and these individuals could be specifically targeted for vaccination in endemic areas. This focal vaccination will save cost as well as side effects from vaccination. It may also be possible to restrict treatment for those infected individuals who are genetically susceptible. This will spare genetically resistant people from unnecessary and potentially toxic side effects.

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