Part I. Leishmaniasis: Aetiology, Epidemiology and Entomology

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Part I.1 Leishmaniasis – The Parasite and its Identification

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Introduction

The scientific study of leishmaniasis can be said to originate with the publication in 1885 of an article by D D Cunningham which dealt with parasites seen in a large skin biopsy from a patient with Delhi boil, isolated in that city and examined by Cunningham in Calcutta. It is clear from his description and drawings that he was describing the cytoplasm of parasitised macrophages containing numerous leishmanial amastigotes and suggesting that what we now know to be the parasite were the spores of a slime mould. Further progress in the 19th century was limited to a confirmation of Cunningham’s observations by Firth (1891), except for an unfortunately obscure report by Borovsky (1898) working in Tashkent, who gave the first accurate description of the cutaneous parasite and its relationship to the host cell. Then in 1903 the pace of discovery quickened with the discovery by Leishman (1903) that there was a specific aetiological agent for the illness not uncommonly contracted at the military camp of Dum-dum near Calcutta, an illness which was at that time thought to be a pernicious form of malaria. Leishman’s idea that the visceral parasites were degenerating trypanosomes was corrected in the same year by Donovan (1903) who observed the same parasite in fresh biopsy material. In the same year yet another milestone was reached with the accurate description of the parasite of Old World cutaneous leishmaniasis by Wright (1903) in America. The following year Rogers (1904) reported the culture of the visceral parasite and Nicolle (1908) cultured the cutaneous parasite in a medium that for more than half a century was the main medium used to culture Leishmania.

Taxonomy of Leishmania

The importance of the availability of culture forms for laboratory investigation cannot be over-emphasised, as was widely recognised in the earliest studies including Cunningham’s paper. By the time that Nicolle had successfully cultured these organisms, both the cutaneous and visceral parasites were known to be widely distributed, with for example the visceral parasite known from India, China, Arabia, and several countries around the Mediterranean, and it was believed that the morphological similarity of all these parasites was masking genetic diversity between these organisms. Despite the optimism of Nicolle who expected the culture forms to provide the differences by which the various types of Leishmania could be distinguished, efforts to find suitable taxonomic characters have occupied a prominent position in Leishmania research for many years. The clinical picture in man, geographical distribution and epidemiological features assumed great importance in the nomenclature of the genus, and it was not until the 1970’s that more satisfactory criteria for the identification and classification of Leishmania were developed.

Of particular significance was the application of a number of biochemical and immunological methods which were predominantly independent of the interaction of the parasite with the environment in either vector or host and which closely reflect the genome of the organism (Chance & Walton, 1982). Interestingly, in parallel with the development of biochemical and immunological techniques there has been a reappraisal of the basic biological characters of Leishmania with even the most basic of characters such as amastigote size giving useful information with clear size differences seen in both the light and electron microscope (Gardener et al., 1977).

Leishmania Epidemiology

The most widely used technique in Leishmania epidemiology is that of isoenzyme electrophoresis, the results of which have made many contributions to our understanding of the epidemiology of both cutaneous and visceral leishmaniasis. A number of different matrices have been used for isoenzyme electrophoresis and until recently starch-gel was the most favoured. However the simplicity and convenience of the cellulose acetate method has recently resulted in considerable attention being paid to the use of this medium. Initial studies of isoenzyme variation in Leishmania were based on the simple visual comparison of the electrophoretic mobilities of relatively few enzymes. However the distribution of genetic similarity in interspecific comparisons make it important that as many genetic foci as possible are sampled, and therefore more recent studies have included between ten and twenty enzymes of a large number of stocks (Le Blancq et al., 1986). Such comparisons are best made using mathematical procedures and computer analysis. Matrices of similarity coefficients between all pairs of stocks can be constructed and then subjected to cluster analysis with the production of dendrograms from which the relationships and groupings of stocks can be readily appreciated (Lanotte et al., 1981). Further analysis using ordination techniques such as principal component analysis is also possible. If electrophoretic patterns are related to particular genetic loci, then the frequency of alleles at a locus may be analysed to give genetic distances or identities over all the loci studied. Genetic distances may also be expressed as dendograms. The
numerical analyses of isoenzyme data provide information of systematic value as opposed to parameters which provide information useful only for identification purposes.

Isoenzyme studies have made several major contributions to the study of leishmaniasis (Chance, 1979). The divisions of the genus can now be delimited with considerable confidence. Within the Old World the separation of the parasites causing cutaneous disease into L. major and L. tropica is now well established, with L. aethiopica not closely related to either. All the parasites causing visceral leishmaniasis, including those in the New World, have proved to be closely related. Within the New World the complexes of L. aethiopica and L. braziliensis have clearly been shown to be separate entities confirming their separation on the biological characters of size and patterns of development in mammalian and invertebrate hosts. Many of these findings are confirmations of previously accepted divisions within the genus; of much greater significance are the contributions made by the ability to identify individual isolates, thus allowing a fuller understanding of epidemiological cycles with the identification of parasites in potential reservoirs and vectors. An important example is the realisation that visceral infections in rodents in Africa are usually L. major and are not therefore reservoirs of L. donovani s.l. as had been previously suspected. We also now have a clear understanding of what type of disease can be caused by each parasite, thus for example L. donovani may cause both visceral and cutaneous disease, and L. tropica may on occasions give rise to visceral infections. Thus we see a re-emergence of the spectral theory of leishmaniasis in which the same parasite may cause a variety of clinical syndromes according to the reactivity of the host.

In addition to the isoenzyme electrophoresis many other biochemical and immunological techniques have been used to characterise Leishmania (Chance, 1985). The two techniques of most current interest are the use of monoclonal antibodies and nucleic acid hybridisation. A major feature of both these approaches are attempts to develop rapid methods of identification based on the use of radioactive, fluorescent, or chromogenic probes for the detection of parasites in biopsy material. Unlike current methods in use, the aim of these methods is to be of immediate use in patient management. Some progress has been made in this area with the use of radioactive kinetoplast DNA probes to identify flagellates in sandfly guts.

Clinical Detection of Leishmania

Indirect methods of detecting the presence of leishmanial parasites, i.e. detecting antibodies to leishmanial antigen have the obvious advantage of avoiding invasive diagnostic methods. Although the sensitivity of present immunological methods is considerable, they are handicapped by problems of specificity. In an attempt to develop and improve antibody detection tests, we have examined the surface of L. donovani for an antigen which elicits a strong response in humans and which does not cross react with antibodies to other organisms, and which preferably, for technical reasons, is present in promastigotes. I labelled promastigotes, surface labelled by the iodogen method were immunoprecipitated with kala-azar serum and run on SDS-PAGE. One major protein band of 63 kilodaltons was detected which was found to be common to all geographic variants of L. donovani and also to other species of Leishmania. This molecule was not immunoprecipitated by human sera against other infectious organisms known to cross react with leishmanial antigen, and would appear to be an ideal candidate for investigation as an immunodiagnostic reagent. Studies in this area are continuing.

There has clearly been a great deal of progress in our understanding of the divisions within the genus Leishmania and the hope for the future is that clinically useful techniques will become available for the rapid detection and identification of Leishmania.

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Part I. II Current Methods in Studies on the Ecology and Epidemiology of the Leishmaniases with Particular Reference to the Americas

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Introduction

Most forms of human leishmaniases are zoonoses, with a primary origin in wild animals, and occasionally involving domestic animals—in particular the dog. Elucidation of the “epidemiological triangle” is the