terial antigen and the patient may then develop what is known as a 'reversal reaction'.

Erythema Nodosum Leprosum (ENL)

In reversal reactions in leprosy the main infiltrating cell is the lymphocyte and the lesion resembles a tuberculin reaction. However, another type of reaction can occur in the skin of patients with lepromatous leprosy. This condition, called erythema nodosum leprosum, develops in lepromatous patients on chemotherapy at a time when massive destruction of the organisms is occurring. The lesions are generally erythematous and indurated, but may become necrotic, pustular or hæmorrhagic. Histologically the lesions are characterized by an intense perivascular infiltration with polymorph leukocytes. The vessels in the centre of the lesions may show fibrinoid necrosis and swelling of their endothelium. The tissues round the damaged vessels are œdematous. The appearance both clinically and histologically is that of an Arthus reaction. Moreover, the patients may have other evidence of immune complex deposition. This includes fever, albuminuria, arthritis and iridocyclitis.

Using the indirect fluorescent antibody technique, it was possible to demonstrate granular deposits of immunoglobulin and complement in a perivascular deposition in the dermis in 10 out of 17 patients examined (Wemambu *et al.* 1969). The distribution of these deposits corresponded to the areas of polymorph infiltration. In a few of the patients examined evidence was also found that there was soluble mycobacterial antigen present in the lesion. The failure to find deposits in 7 patients examined is consistent with the observation in experimental animals that no such deposits can be found in Arthus reactions examined 24 hours after their induction. A further observation of interest is that extremely high serum levels of the third component of complement (over 2·5 mg/ml) were found in some of the patients with ENL. High levels of the second component have also been described in this condition (Saitz *et al.* 1968).

ENL is therefore due to a hypersensitivity reaction against free mycobacterial antigen, produced by the deposition of immune complexes containing antibody and complement in the tissues. This occurs under conditions where specific cell-mediated immune processes have been suppressed but humoral antibody is present in high concentration in the circulation.

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Immunological Aspects of Clinical Leishmaniasis

The prototype of infections caused by leishmania is simple cutaneous leishmaniasis (oriental sore) which is a self-healing disease. Some weeks after inoculation of *Leishmania tropica* by a sandfly of the genus *Phlebotomus* a nodule appears in the skin (Rahim & Tartar 1967). After a period varying from 2 to 8 months this ulcerates and is covered by a crust. The lesion may then remain almost unchanged for up to a further 12 months, when it starts to heal slowly, leaving in the end a characteristic depressed mottled scar.

For some years it has been realized that cutaneous leishmaniasis presents a spectrum of disease similar to that seen in leprosy (Destombes 1960). The extreme ends of the spectrum are not as common as in leprosy and are seen only in certain geographical areas, which might suggest that differences in host genetics, parasite strains or epidemiological events influencing host-parasite contact may affect clinical patterns of disease. But there is evidence that many of the clinical features of the disease in an individual patient reflect, or are determined by, his cell-mediated immune response to the parasite.

At one end of the spectrum lies lupoid or recidiva leishmaniasis. The story is of a simple oriental sore that never quite healed or healed and relapsed (Dostrowsky 1936); classically there is a typical

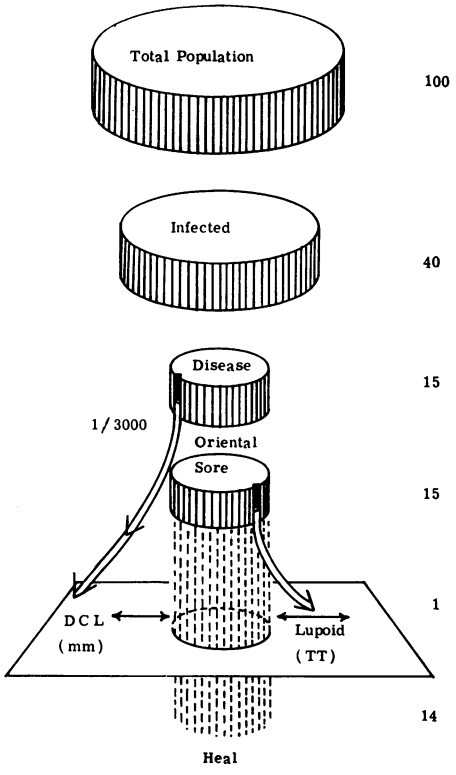

Fig 1 *Spectrum of non-healing cutaneous leishmaniasis seen against the normal course of self-healing oriental sore. Numbers indicate estimated relative numbers of people in each group in endemic areas in Ethiopia (Bryceson 1969, Lemma* et al. *1969). DCL, diffuse cutaneous leishmaniasis.* mm, *macrophage.* TT, *tuberculoid histology*

scar around whose borders small nodules recrudesce, ulcerate and heal so that the whole lesion spreads locally slowly but never metastasizes; there are many possible variations. Histologically the lesion shows an intense cellular response in the form of a tuberculoid granuloma without caseation; parasites are scanty or absent, but the presence of unusual numbers of plasma cells may suggest the diagnosis (Petit 1962). The condition is associated with marked delayed hypersensitivity on intradermal skin testing with leishmanin and, sometimes, an Arthus reaction (Bray *et al.* 1967).

At the other end of the spectrum lies diffuse cutaneous leishmaniasis (DCL) which has been described from South America, notably by Convit (1958) in Venezuela, and from Ethiopia (Price & FitzHerbert 1965). The story is of a primary nodule which does not usually ulcerate or heal but spreads locally after a period of weeks or months and also metastasizes to other parts of the skin. The histology shows a mass of heavily parasitized macrophages and a remarkable absence of small lymphocytes and epithelioid cells although plasma cells are usually present (Bryceson 1969). Despite the size of these nodules, there is no epidermal reaction or ulceration. Cutaneous hypersensitivity to leishmanin is absent. This 'anergy' is specific as responses to lepromin and tuberculin are normal. There may, however, be some general deficiency of cellular immunity as only 2/8 patients could be sensitized to DNCB. Relapse almost invariably follows treatment.

Similarities with lepromatous leprosy in each of these characteristics are obvious. Moreover, there is a precise parallel between the histological spectrum from tuberculoid to lepromatous in leprosy (Ridley & Jopling 1962, 1966) and that from tuberculoid to macrophage in leishmaniasis (Bryceson 1969).

Fig 1 illustrates this spectrum of non-healing disease, arrested on a platform in time, in relation to the more usual progression towards healing of simple oriental sore. Horizontal movement along the spectrum is suggested by a number of observations. Progression towards DCL can come in waves associated with systemic illness (Price & FitzHerbert 1965) which may represent periods of diminished immunological activity. Reaction states have also been described and these may be of two types: One, characterized by fever and local flare up of lesions, is associated histologically with polymorphonuclear leukocyte infiltration (Destombes *et al.* 1965) and may be a form of Arthus reaction similar to erythema nodosum leprosum (Wemambu *et al.* 1969). The other is characterized by acute swelling and coalescence of early lesions, is associated with œdema but not polymorphonuclear leukocyte infiltration (Bryceson 1969) and may be a form of reversal reaction (Ridley 1969). Progression towards the tuberculoid end is shown by patients with DCL under the influence of prolonged treatment (Bryceson 1970) who may, in relapse, come to show an intermediate or tuberculoid histology and a positive leishmanin test. It is interesting that without this immunological shift it is very difficult to cure the patient. It is not clear whether it is necessary for a patient with lupoid leishmaniasis to move away from the tuberculoid pole before his lesion can heal. Two observations support this idea. The first is that treatment with systemic steroids combined with antimonals is more effective than antimonals alone (Evan-Paz & Sagher 1961); the second is that intralesional steroid alone cures the condition.

In order to study the immunological aberrations that underly these polar forms of disease it is necessary first to understand the processes by which healing takes place in oriental sore and by which immunity is established. The clinical observations, known for a long time, can be briefly

summarized. In all forms of leishmaniasis healing is accompanied by delayed hypersensitivity and is followed by long-lasting immunity to the homologous organism. In South American cutaneous leishmaniasis, including the chronic metastasizing form, espundia, delayed hypersensitivity develops early in the infection (Adler 1963), but in visceral leishmaniasis which is usually fatal if untreated it does not appear until after drug-induced cure (Manson-Bahr 1963).

Antibodies have been demonstrated only occasionally in patients with oriental sore in Iraq (Bray & Lainson 1967). It is interesting that the intense transmission of urban leishmaniasis, due to *L. tropica* var. *minor*, in certain Middle Eastern towns is associated with exquisite delayed hypersensitivity, Arthus reaction and relatively high incidence of lupoid disease; while the rural zoonotic form, due to *L. tropica* var. *major*, which is transmitted relatively infrequently to man is more florid, more rapidly healing and is seldom lupoid (Manson-Bahr 1964). There have, however, been no studies on the relationship between healing, chronicity and antibody levels. In Ethiopian DCL low titres of hæmagglutinating antibodies comparable with those found in local oriental sore are present and suggest that the immunological defect is confined to cell-mediated mechanisms. Antibodies are not regularly present in simple South American cutaneous leishmaniasis or DCL; but in espundia high titres may be shown by immunofluorescence (Convit & Kerdel-Vegas 1965) or indirect hæmagglutination (Bray & Lainson 1967). Antibodies can, however, be detected in high titre in visceral leishmaniasis by any of the conventional methods, and there are very high levels of IgG and IgM (Chaves & Ferri 1966) which probably contain only a small fraction of specific antileishmanial antibodies (Garnham & Humphrey 1969). The fall in antibody titre in this disease is associated, in time, with the emergence of delayed hypersensitivity. Some of these facts are summarized in Table 1. Antibody seems to be associated more with failure to heal than with healing. It seems likely that one immunological disorder, namely specific depression of cell-mediated immunity, is common to DCL and kala-azar, and that the differences are due largely to different preferred sites of parasite multiplication. The failure to develop cell-mediated immunity, as shown by the negative skin test during the incubation period in kala-azar (Manson-Bahr, personal communication) and the absence of ulceration in early DCL, is of interest in view of the long incubation period or time till spread, respectively, in these diseases (Manson-Bahr & Southgate 1964, Bryceson 1969). It suggests that the organism either fails to induce a primary immunological response by avoiding contact with immunologically competent cells or by disguising itself in some way, or else it actually paralyses the response at a very early stage, by a mechanism akin to low zone tolerance. That organism and host are, respectively, antigenically and immunologically competent becomes apparent as the conditions regress under treatment.

Table 1

Features of polar forms of cutaneous leishmaniasis

Features	*Diffuse cutaneous leishmaniasis (DCL)*	*Lupoid or recidiva leishmaniasis*
Clinical	Disseminated nodules	Local scar with peripheral spread
Histology	Parasitized macrophages (MM)	Tubercles (TT)
Delayed hypersensitivity to leishmanin	Negative	Positive, occasional Arthus
Antibodies	Variable	Variable
Immunoglobulin	Normal	(not done)
Response to treatment	Poor, relapses	Variable, improved by steroids

MM, macrophage. TT, tuberculoid (Bryceson 1969)

The next clinical observation is that of the 'isophasic response': when a patient with oriental sore or lupoid leishmaniasis is challenged with the homologous strain a lesion develops which is histologically similar to and heals with the primary lesion (Adler 1963). That is to say development of immunity to reinfection does not precede healing. In espundia, however, immunity to reinfection precedes healing, suggesting that organisms of the primary infection are in some way protected from a competent immunological response.

Studies on cross-immunity between strains suggest that some strains, e.g. *L. tropica* var. *major* (Adler 1963), may be more immunogenic than others against which they protect, e.g. *L. tropica* var. *minor* (Manson-Bahr 1964). This antigenic distinction can be shown qualitatively by Adler's technique in which the strains are grown in the presence of specific immune rabbit sera, or quantitatively by a hæmagglutinating technique using cross-absorbed rabbit antisera. By this method Bray & Rahim (1969) showed that Iraqi strains of *L. tropica*, which do not cause DCL, have more antigens than do Ethiopian strains, which can cause DCL. All Ethiopian strains, whether from oriental sore or DCL, are, however, indistinguishable by this technique (Bray & Bryceson 1969) confirming that it is not simply the antigenic character of the parasite that causes DCL.

It is, however, clear that our understanding of these problems cannot proceed much further without a good experimental model. Such a model exists in the infection of the guinea-pig with *L. enriettii*, its natural parasite (Bryceson, Bray, Wolstencroft & Dumonde, 1970). Under normal conditions a single cutaneous lesion is produced which heals within 8 – 10 weeks. This is accompanied by delayed hypersensitivity and by *in vitro* evidence of cellular immunity, namely

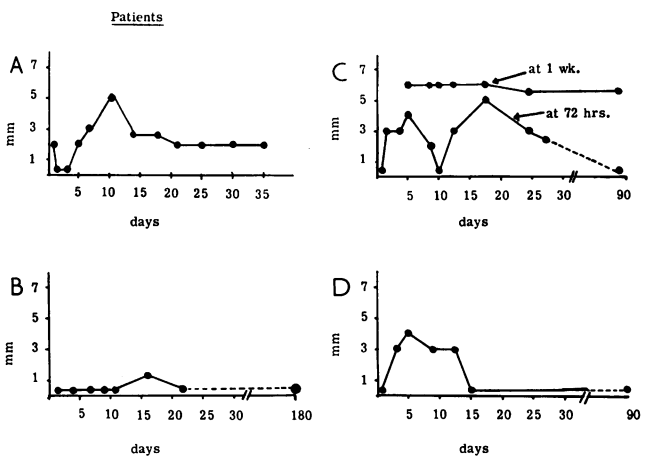

Fig 2 *Leishmanin sensitivity of 4 patients with DCL after receipt of 2·5–4 × 10⁷ lymphocytes from leishmanin-sensitive donors. (Reproduced from Bryceson 1970 by kind permission)*

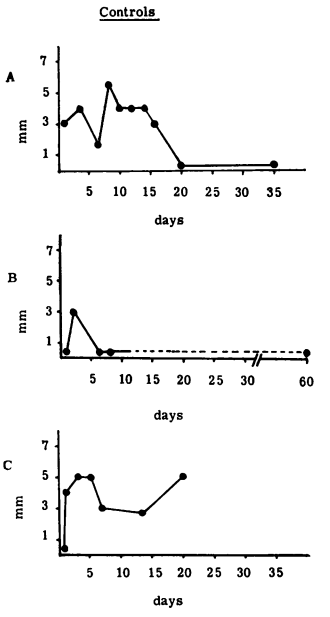

Fig 3 *Leishmanin sensitivity of 3 healthy volunteers after receipt of 4–8 × 10⁷ lymphocytes from leishmanin-sensitive donors. (Reproduced from Bryceson 1970 by kind permission)*

transformation of lymphocytes with specific antigen, inhibition of peritoneal macrophage migration, the production of 'lymphokine' factors and the destruction of target cells (monolayers of parasited macrophages) by specifically and also, interestingly, nonspecifically sensitized lymphocytes from animals immunized with Freund's complete adjuvant (Dumonde *et al.* 1969). It is, however, proving difficult to define conditions in which macrophages can retard the growth of parasites; and the way in which the guinea-pig eliminates the parasite is not yet established. In this infection antibodies can be detected by immunofluorescence by 2 weeks, soon after the development of delayed hypersensitivity (Radwanski, Bryceson, Dumonde & Bray, in preparation), and anaphylactic antibodies can be detected at about 8 weeks which is when healing begins. In this model it has also been possible to mimic the isophasic response, to demonstrate immunity in the face of progressing metastatic disease, and to produce a condition closely resembling DCL in man.

Table 2

Preliminary studies of human lymphocyte responses to *in vitro* incubation with leishmanial antigen

Donor skin sensitivity		*Lymphocyte transformation*	*Mitogenic activity of supernatant*	
Leishmanin	*PSA*		*Autologous cells*	*Homologous cells*
6 pos.	5/5 pos.	5/6	2/3	2/3
4 neg.	0/3 pos.	1/4	1/3	1/3

Numbers refer to numbers of patients tested.
PSA 'purified' protein-rich leishmanial antigen; positive to 1 μg or negative to 100 μg.
Lymphocyte transformation measured by ^{3}H thymidine uptake.
Supernatant of lymphocytes incubated for 3 days with 100 μg PSA, membrane filtered and cultured with fresh lymphocytes

Experimental studies of cell-mediated immunity to leishmaniasis in man are at an early stage. As long ago as 1965 Adler & Nelken attempted but failed to transfer delayed hypersensitivity to leishmanin in man, using peripheral leukocytes. It is possible that the challenge dose of antigen was inadequate (10^5 organisms). In attempts to induce immunological competence in patients with DCL I obtained peripheral lymphocytes from 4 leishmanin-sensitive donors and injected them intradermally into 4 leishmanin-negative patients and 3 leishmanin-negative normal volunteers (Bryceson 1970). Figs 2 and 3 show that in several cases tranfer of delayed hypersensitivity (to 5×10^6 organisms) was achieved for a few days. In no instance did this affect the course of the disease. The dangers of transferring serum hepatitis or of causing graft-versus-host disease, however, probably outweigh any possible benefits of this form of therapy.

In vitro studies of cutaneous leishmaniasis in man, carried out with Dr R A Maini and Dr Dumonde, have shown the phenomena of lymphocyte transformation in the presence of specific antigen and the production of mitogenic factor ('lymphokine') in supernatants of sensitized cells incubated with that antigen. Table 2 illustrates our preliminary results. It will be seen that there is by no means perfect correlation between delayed hypersensitivity and lymphocyte transformation. It is, however, not always possible to know pre-

cisely with which strain a patient was infected and there is some suggestion that this test may reveal specificity not apparent in skin testing. Detailed analysis of leishmanial antigens is now necessary if we are to understand the problems of sensitization, immunity, cross-protection and specific immunosuppression. This, together with elucidation of how the host overcomes the parasite in the experimental model of oriental sore may enable us to find out what has gone wrong with those patients with mutilating or lethal disease who lie at the ends of the spectrum.

In interpreting the results of immunological studies in terms of disease patterns in leishmaniasis it will, however, be necessary to take into consideration the interface between immunology and epidemiology, which determines equally important variables including parasitic variation, susceptibility of host and repetition of antigenic challenge.

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Immunological Aspects of Experimental Leprosy in the Mouse

It is widely accepted that immune responses of the cell-mediated, rather than of the humoral, type play a major part in determining the course of disease in human subjects exposed to infections with *Mycobacterium tuberculosis*. Therefore it has been assumed that similar immunological responses determine the outcome of infections with *Mycobacterium leprae* in man. Moreover, in human leprosy, there is a wide variety of clinical forms which range from the tuberculoid, with presumed high resistance, to the lepromatous, with presumed low resistance. In the last few years, these assumptions have been submitted to more detailed analysis using available immunological techniques. The results support the contention that cell-mediated immune processes determine the state of infections with *M. leprae* in man.

Before 1960, no contributions from experimental infections were possible, because until then infections with *M. leprae* had not been obtained in animals. In the last 10 years, the mouse foot-pad infection (Shepard 1960, Rees 1964) has been exploited and the various techniques which have been applied to enhance these infections with *M. leprae* have shown the importance of the immunological capacity of the mouse in determining the outcome of the infection and the resulting disease process.

Experimental leprosy in the normal mouse: Mice of the same genetic stock (CBA), age and sex (female) were used throughout, thus obviating any genetically-controlled differences in their susceptibility to infection or their immune responses. Following local inoculation of *M. leprae* in the foot-pads of normal mice, there was an initial logarithmic phase of local multiplication of bacilli lasting for 6–8 months. Subsequently, the rate of multiplication diminished, and the proportion of degenerate bacilli increased throughout the life span of the animal. Up to 15 months, none of the histological features of this infection were typical of human leprosy, although at this later period bacilli were found in dermal and peripheral, particularly sciatic, nerves. However, two years and more after inoculation, epithelioid granulomata and overt neural damage were found at the sites of inoculation (Rees *et al.* 1969, Rees & Weddell 1970). Histopathologically these were in every detail comparable with lesions found in patients