Experiences with vaccines against cutaneous leishmaniasis: of men and mice

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SUMMARY

The need for a vaccine(s) against cutaneous leishmaniasis and the populations at risk for whom such vaccines should be developed are briefly discussed. The current human vaccine studies are reviewed, as are some experimental mouse studies with emphasis on *Leishmania major* infection relevant to vaccine development. Based on the information available from the mouse model and those data which are being sought in human studies, the benign nature of the cutaneous disease, the ease with which *L. major* can be manipulated to yield the required material, and the ongoing practice of leishmanization which allows rapid evaluation of candidate vaccine(s), it is suggested that a vaccine, at least against *L. major*, is imminent in the not too distant future.

Key words: cutaneous leishmaniasis, vaccines, *Leishmania major*, humans, mice.

INTRODUCTION

It was the observation that milkmaids who recover from cowpox are immune to infection with smallpox that prompted Jenner to perform his bold human experiment. Subsequently, cowpox virus ('vaccinia') became a vaccine against smallpox and the term 'vaccination' was applied to the general practice of immunization against an infective agent. Similarly, the term 'leishmanization' has recently been applied to an ancient practice of deliberate infection with *Leishmania* for the purpose of inducing a long-lasting immunity against Old World Cutaneous Leishmaniasis (OWCL) which often involves multiple lesions in exposed areas of the body.

Clearly, this method of prophylactic immunization is not without risk and difficulties, as will be discussed later. However, in the absence of any other feasible or practical control measures, leishmanization was used in the past in the USSR (Kellina, personal communication) and Israel (Greenblatt, 1980, 1988) and is being used on a large scale in Iran today (Nadim & Javadian, 1988).

There are several excellent review articles on immunological studies of experimental leishmaniasis (Behin & Louis, 1984; Liew, 1986; Blackwell, McMahon-Pratt & Shaw, 1986; Mauel & Behin, 1987; also see a collection of articles edited by Louis & Milon, 1987; Muller, Pedrazzini, Farrell & Louis, 1989). This report will therefore focus primarily on ongoing human studies and those of the *L. major* mouse model as they pertain to vaccine development.

WHO NEEDS A VACCINE?

An important issue to be considered for vaccine development is the population for whom vaccines are required. At present chemotherapy with antimonial drugs is expensive, treatment is required for a long time, produces side-effects, and is associated with numerous relapses and refractory cases. Indeed, it is not recommended to treat the zoonotic OWCL cases caused by *L. major*, unless multiple disfiguring or non-healing lesions are seen. The patients have no choice but to accept the suffering from a 6–9 month open ulcer(s) and the consequences of the non-aesthetic scar(s) which are left for the remainder of their lives. On the other side of the spectrum, visceral leishmaniasis (VL) of the Old World caused by *L. donovani* is presumably lethal if untreated once the signs and symptoms of the disease are well developed. In the visceral leishmaniasis (VL) caused by *L. chagasi* (and possibly *L. infantum*) the infection may be subclinical and self-healing in a relatively large number of cases. In certain individuals, perhaps due to factors like malnutrition, the infection will develop into a progressive disease which will require treatment (Badaro, Jones, Carvalho, Sampaio, Reed, Barral, Teixeira & Johnson, 1986*b*; Badaro, Jones, Lorenço, Cerf, Sampaio, Carvalho, Rocha, Teixeira & Johnson, 1986*c*). Subclinical infection with *L. donovani* has also been recognized by Sacks, Lal, Shrivastava, Blackwell & Neva (1987).

Amongst the most difficult cases to cure, are the mucocutaneous (MCL) patients who cannot be definitively cured even with high doses of antimonials given for prolonged periods (Marsden, 1986).

Not knowing all the risk factors involved in the development of VL and MCL following the infection, it is difficult to know whether a vaccine

should be used for a general population living in an endemic zone. The risks involved in injection of foreign material as a vaccine to healthy individuals, particularly children, must be carefully evaluated against the risks of getting the disease and the possible effects thereof. In addition, one must weigh the feasibility and cost of other control measures (to decrease the risk of disease) against those of vaccination. So far, with the limited information available, it seems that vaccination would be the most practical control tool for certain populations, if safe, effective and inexpensive vaccines were available. Certain groups have predictably a very high risk factor, for example, workers on new development projects, individuals from non-endemic regions who move to a highly endemic area, and children in hyperendemic regions where almost all are eventually infected. This is particularly true for zoonotic OWCL. These are obvious targets for vaccination but others must be carefully evaluated.

HOW MANY VACCINES ARE NEEDED AGAINST LEISHMANIASES?

Leishmaniasis is often referred to as a 'spectral' disease, because of the different clinical or pathological forms in which it can be presented. Indeed, some of the major forms (visceral and cutaneous) may be considered as different diseases, for they are normally caused by different species with completely different pathology, vector and reservoir specificity and ecology. Common features to all forms of leishmaniases are (*a*) large number of common antigens shared by different species, (*b*) all are transmitted by female sandflies, (*c*) the sole target cell in the mammalian host is believed to be the macrophage and (*d*) generally, resistance to re-infection by the same organism follows a successful recovery from the original infection.

Despite the considerable antigenic cross-reactivity amongst *Leishmania* species, recovery from an infection does not confer immunity against others. However, some cross-protection has been noted, e.g. human infection with *L. major* is believed to produce a protective response against *L. tropica* infection (but not vice versa) and some experimental cross-reactivity has been demonstrated (reviewed by Mauel, 1982; Alexander & Kaye, 1985; Mitchell & Handman, 1987; Alexander, 1988).

Conceivably, a vaccine using some common antigens of the parasites might be effective against all forms of leishmaniases provided that they are presented by the appropriate antigen-presenting cells (APC) and can induce the protective T cell response. Unfortunately the characteristics of the antigens of different species of *Leishmania* presented on the surface of APC are not known.

Alternatively, an immune response against the common sandfly vectors which can enhance the virulence of the inoculum, as described recently (Titus & Ribeiro, 1988) may induce a transmission blocking immunity which could be effective against several forms. A factor has been identified which closely resembles one of the peptides of the calcitonin-related gene products (CRGP). This peptide produces a strong vasodilation and local inflammatory reaction, thereby providing monocytes, target cells for *Leishmania* (Titus and Ribeiro, manuscript submitted). These points are highly theoretical and require further investigation.

VACCINE DESIGN

One might consider that most, if not all, effective vaccines used in humans so far, have been developed without knowing details of the mechanisms of protection, or the nature of the protective antigen (epitope). The empirical approach of vaccine development, therefore, should not be totally abandoned until vaccine design can be accomplished with the new technology.

The Scientific Working Group on Leishmaniases of the UNDP/World Bank/WHO Special Programme on Research and Training in Tropical Diseases (TDR) recommended that the Programme should simultaneously pursue several approaches, e.g. empirical approach using killed organisms for prophylactic immunization (Mayrink, Williams, Da Costa, Magalhaes, Melo, Dias, Lima, Michalick, Carvalho, Barros, Sessa & de Alencar, 1985; Antunes, Mayrink, Magalhaes, Costa, Melo, Dias, Michalick, Williams, Lima, Vieira & Schettini, 1986), and with BCG, for immunotherapy (Convit, Castellanos, Rondon, Pinardi, Ulrich, Castes, Bloom & Garcia, 1987), semi-purified, or well-defined preparations showing promise in animal studies (Monjour, Ogunkolade, Pointet & Vouldoukis, 1985; Handman & Mitchell, 1985, 1987; Monjour, Ogunkolade, Vouldoukis, Roseto, Berneman & Frommel, 1986; Frommel, Ogunkolade, Vouldoukis & Monjour, 1988; Russell & Alexander, 1988; Scott, Pearce, Nativitz & Sher, 1987) and genetically engineered vaccine constructs which still require some fundamental development.

WHAT KIND OF VACCINE IS NEEDED?

An important requirement for many vaccines is that they must prevent infection. This may not be necessary for a vaccine against leishmaniasis, as discussed below, infection may exist without an observable pathological manifestation and disease prevention without interfering with infection may be an advantage.

Experiments in mice of different genetic background infected with *L. major* (Leclerc, Modabber, Deriaud & Chedid, 1981) indicated that the organism remains viable in various organs long after the lesion

is healed. One possible mechanism by which the parasites can evade the immune responses of the host is that they are able to hide within the 'safe targets' (pre-granulocyte–macrophage cells), which increase considerably during infection (Mirkovich, Galelli, Allison & Modabber, 1986; Modabber, 1987). These cells presumably cannot be activated to kill the parasite (Hoover & Nacy, 1984).

In humans, cell-mediated immune responses persist for many years following recovery from an infection (Wyler, Weinbaum & Herrod, 1979). In my personal case a very strong delayed-type hypersensitivity (DTH) reaction to leishmanin as measured by skin test, was observed 14 years after living in a non-endemic area (almost 24 years after 3 lesions had healed). This strongly suggests that antigens or parasites persisted for a very long time after recovery from the infection. Furthermore an increasing number of patients with acquired immune deficiency syndrome (AIDS) or other form of immune deficiency are being reported also to suffer from recrudescent leishmaniasis (Badaro, Carvalho, Rocha, Queiroz & Jones, 1986*a*; Martinez-Fernandez, Diaz, Sanchez, Carmena, Varela & Navas, 1987; Verdejo, Alvar, Polo & Lahoz, 1987; Clauvel, Couderc, Belmin, Daniel, Rabian & Seligman, 1986; Medrano-Gonzalez, Lorenzo & Perez, 1986; Senaldi, Cadeo, Carnevale, Di Perri & Carosi, 1986; Franco-Vicario, Heredia, Rojo & Hermosa, 1987; Mulliez, Dabouz, Demory, Darras & Crinquette, 1987; Antunes, Carvalho, Tavares, Botas, Forte, Del Rio, Dutschmann, Costa, Abranches, Pereira, Paiva, Araujo & Baptista, 1987). It has been suggested that leishmaniasis should be considered as an opportunistic disease (Badaro *et al.* 1986*a*).

All these observations indicate that at least in some cases, leishmaniasis produces a state of premunition (concurrent infection and immunity) which may be the underlying mechanism for prolonged immunity to reinfection generally seen in recovered individuals. Only when the immune response becomes deficient can the disease relapse.

If this is true, then the vaccine does not have to prevent infection to be disease-protective for the general population. This reduces the stringent requirements for a protective vaccine. In any case, for those living in an endemic region where they are continuously exposed to leishmanial antigens even a vaccine which can produce a short-term immunity may be sufficient when given at an appropriate time.

The degree of immunity is generally related to the dose of the virulent parasite given; while a low dose may be tolerated without producing a lesion, a high dose will overwhelm the immune response. Hence a vaccine which can only reduce the effective dose of the inoculum may be adequate for some populations who develop some degree of natural immunity as a result of repeated exposure to subinfective doses of the parasite.

On the other hand, repeated infection with the same organism has been reported in a few cases (e.g. Killick-Kendrick, Bryceson, Peters, Evans, Leaney & Rioux, 1985). This may represent an aberrant immunological response induced by previous infections, genetic or other factors. Indeed, repeated infection may occur but it is usually milder and recovery is quicker. It must be noted that in highly endemic regions, following recovery from a natural infection, repeated infections are rare, at least in the ZCL area.

Cutaneous leishmaniasis is the most frequently occurring form of the disease and this is the form which probably imposes the highest economic loss, particularly in new development projects in the Latin American countries and the Middle East. As the disease is relatively benign and the immunity produced following recovery from the original infection is generally life-long for indigenous populations, the development of a vaccine seems the least difficult compared to vaccines against other forms. In certain populations of the ZCL-endemic areas, almost 100% will eventually become infected and age-related incidence remains predictably constant for children. This, in addition to the programmes of leishmanization, provides a special opportunity for evaluation of candidate vaccines. All these points indicate that a prophylactic vaccine against ZCL is feasible, beneficial, cost-effective and needed for those who live in or enter hyperendemic zones of OWZCL.

Development of a vaccine(s) against other forms of the disease, however, is not as simple and requires much more detailed information on the pathology of the disease, risk factors, etc.

An inexpensive vaccine which produces a life-long protection after a single administration without any side-effects would be ideal. Live vaccines which establish a state of premunition require one administration and produce a long-lasting protection. As *Leishmania* produce a state of premunition (shown in animal models and implied by the recrudescent VL in AIDS, patients without previous history of clinical leishmaniasis as mentioned above), a live vaccine should not be ruled out despite disappointing trials in the past (Heyneman, 1971).

The failure of previous vaccine trials has been attributed to changes of the parasite in culture, as indicated by Mauel (1982). The stability of the parasite must be assured in any vaccine development strategy. Changes of the characteristics of the parasites in culture have been re-examined elegantly for many *Leishmania* species in recent years (Giannini, 1974; Keithly & Bienen, 1981; Sacks & Perkins, 1984; Franke, McGreevy, Katz & Sacks, 1985; Wozencraft & Blackwell, 1987; Melo, Williams, Magalhaes-Rocha, Baba, Mayrink, Michalick,

Da Costa, Diaz & Magalhaes, 1987; Turco, Hull, Orlandi, Sheperd, Homans, Dweck & Rademacher, 1987; Howard, Sayers & Miles, 1987; Celeste & Guimaraes, 1988), and inhibition of one species by another in a mixed culture has also been seen (Pacheco, Grimaldi & Morel, 1987).

In addition, the isolate used for vaccination was not cloned as methods for cloning had not been developed and the possibility of a mixed *Leishmania* culture being used as the original vaccine preparation cannot be dismissed.

In recent years, biochemical and genetic analyses of *Leishmania* spp. have provided a large number of powerful tools and markers which can be used for characterization and identification of parasites, and to assure the stability of vaccines, whether a live or non-living vaccine is used (Arnot & Barker, 1981; Wirth & McMahon-Pratt, 1982; Jaffe & McMahon-Pratt, 1983; Etges, Bouvier, Hoffman & Bordier, 1985; Blackwell, 1985; Chang, Inserra, Kink, Fong & Chang, 1986; Button & McMaster, 1988; Puentes, Sacks, Da Silva & Joiner, 1988).

The encouraging view of the prospects for a live vaccine is based on experimental vaccination of mice with non-pathogenic live clones of *L. major* (Mitchell, Handman & Spithill, 1984). The genetically engineered organism carrying genes of protective antigens from different pathogens may not be as far-fetched as it seemed a few years ago (Jacobs, Tuckman & Bloom, 1987).

HUMAN VACCINATION

At present there are 3 ongoing studies in human leishmaniasis. These employ living *L. major* (leishmanization, Nadim & Javadian (1988)); a mixture of killed whole organisms (Mayrink *et al.* 1985; Antunes *et al.* 1986) and killed organisms plus BCG (Convit *et al.* 1987). The last is being used for immunotherapy although it may be considered in the future for prophylaxis.

These and the recent information gained from studies on experimental animals are the basis for renewed interest in pursuing vaccine development.

LEISHMANIZATION

The Iranian group has admitted to using leishmanization because all other attempts to control the disease in some communities had failed. As a result of the success obtained in these communities leishmanization was then used as a control method on a large scale because of the very high incidence of ZCL in and around the war zone border of Iran and Iraq. Recruits and volunteers were given the live inoculum about 3 months before being sent to endemic areas. The inoculum, approximately $2–3 \times 10^5$ *L. major* in 0·1 ml of supernatant fluid of 10–15 day NNN cultures was injected intradermally. The inoculum therefore contained all the material present in the supernatant fluid of the medium, e.g. components of rabbit blood, agar, and any product of the parasite.

Table 1. Leishmanization in Iran

(Data from Nadim & Javadian (1988).

Recipient:	1 200 000 Soldiers 160 000 Civilians around Isfahan 60 000 Civilians, war refugees in Khuzestan
Vaccine:	$2–3 \times 10^5$ live virulent *Leishmania major* (lesion of 5–10 mm for 4–6 months). 2–3 % large and non-healing lesions, requiring treatment.
Evaluation in some selected villages (1982). Vaccinated* (530), 14 infected† (2·6 %); non-vaccinated (1724), 250 infected (14·15 %).	

* 77 % takes.

† Lesions were smaller and healed quicker than infected individuals of non-vaccinated populations.

Skin test conversion = 93 % in 'takes', 54 % in 'non-takes' from another study.

According to the report of Nadim & Javadian (1988), over 200 000 civilians and 1·25 million soldiers have received leishmanization within the past 6 years. The analysis of data is incomplete (see Table 1). However, it is reported that out of 8000 cases from the Isfahan area, 'less than 100' had received leishmanization. In general, those who had received inoculation had smaller lesions which healed sooner. In other independent studies, 77 % of inoculated individuals developed a lesion due to inoculation (vaccine takes) and of these 93 % were shown to produce a positive skin test. The skin test was positive in 54 % of non-takes (those who were inoculated but did not develop a lesion).

The rate of skin test positivity in a normal population of age- and sex-matched children was not reported, but it must be much lower than this.

Needless to say this is not an acceptable control method except in extremely critical situations in which the incidence is very high and other methods are impracticable or ineffective. The 'vaccine' itself produces a lesion of 5–10 mm in diameter which lasts an average of 4–6 months, if no complication is produced. This is more than an acceptable side-effect of many vaccines. Complications include large non-healing lesions which may last for years, requiring treatment, and immediate type hypersensitivity reactions which may last for a few hours with or without treatment. As the leishmanization programme introduced individuals with an active lesion to areas where CL was not present before (soldiers returning home) and some indigenous cases were subsequently reported, the question of initiating new foci of transmission has been raised by local authorities. If true (it seems unlikely), it would mean that for *L. major*, human–sandfly–human

Table 2. Killed disrupted parasites

(Two groups were analysed in 1981; only group 1 is presented. In group 2, the difference in incidence was not significant. In the 1983 study, the numbers were too small and the difference was not significant. There was no indication of exacerbation in previously immunized individuals in any of the studies. Data from Antunes *et al.* (1986).)

	Skin test	Total	Cases	Incidence (%)
1981 trial				
Vaccinated	Converted	104	3	2·9
	Non-converted	207	22	10·6
Placebo	Non-converted	289	32	11·1
1983 trial				
Vaccinated	Converted	415	1	0·2
	Non-converted	195	3	1·5
Placebo	Non-converted	616	8	1·3

transmission may occur. More detailed analysis of the cases, the sandfly and particularly the parasite and reservoir hosts is required to establish this.

KILLED LEISHMANIA OF THE NEW WORLD

After the historical studies using killed *Leishmania* in the New World, Mayrink and his colleague (1985) initiated a series of experiments which are the basis of renewed interest in pursuing the development of killed vaccine. As will be discussed later, based on observations in mice, there was a theoretical concern that in humans as well, the injection of killed organisms may induce an exacerbating disease upon subsequent infection. Although the Brazilian studies were not decisive on the protective efficacy of the vaccine, they established an important parameter that, at least in the few infected cases and under the experimental conditions used, there was no exacerbation produced as a result of previous injection of killed organisms.

A summary of the two trials is shown in Table 2, only for the groups which had a statistically significant difference in their incidence rates. The incidence rates of the infection in both studies are very low for evaluation of the efficacy of the vaccine. The trial of 1981 was complicated by a proximal vaccination (in time) with yellow fever vaccine which may have had an immunosuppressive effect, possibly accounting for the low rate of skin test conversion of 33% as compared with the trial of 1983 which produced a conversion rate of 84–90%, depending upon the type of vaccine used. Although the skin test reactivity does not have a causal relationship with protection in mice, analysis of the data from 1981 studies by Antunes *et al.* (1986) suggests that the skin test in humans may be an important indicator of an immune response which fortuitously reflects a state of protection.

Table 3. Immunotherapy compared to chemotherapy

(Data from Convit *et al.* (1987).)

		Cure rate (34 weeks) (%)	Side-effects (%)	
Immunotherapy	(52)	94	5·8	(mild)
Chemotherapy	(42)	94	52·4*	

Vaccine – *Leishmania mexicana* (killed) + BCG $6{\cdot}4 \times 10^8$ + varying doses of BCG.

Drug – Glucantime (50 mg/kg) 20 days × 3, 10 days rest in between.

* Cardiovascular disturbance, bone and muscle pain, hypertension and severe colitis.

The results of Brazilian studies open an avenue for further investigations where the incidence of the disease is high and an unequivocal result on the efficacy of killed vaccines may be obtained.

KILLED LEISHMANIA PLUS BCG FOR IMMUNOTHERAPY

Following the success of immunotherapy of patients with leprosy, (Convit, Aranzazu, Ulrich, Pinardi, Reyes & Alvarado, 1982), a similar procedure was used for treatment of localized cutaneous leishmaniasis, using killed *L. mexicana* with BCG. This treatment was compared with chemotherapy using meglumine antimonate (Glucantime). The results from the first series of patients (Convit *et al.* 1987, see Table 3), indicate that 3 vaccinations over a course of 32 weeks gave a similar rate of cure as was achieved with chemotherapy, but resulted in considerably fewer severe side-effects. It was suggested that immunotherapy is an inexpensive, low-risk alternative to chemotherapy. These studies have now been expanded and the effect of BCG vaccination alone has been shown to be less dramatic (about 41% at 33 weeks as compared to 95% with combination immunotherapy (Convit *et al.*, manuscript submitted)). In addition, the immune responses generated following immunotherapy are being analysed with the aim of identifying the protective mechanism(s) induced by vaccination.

ANIMAL MODELS

Many animal models have been used for immunological and therapeutic studies such as; the hamster with most *Leishmania* species; guinea-pig with *L. enriettii* (see Behin & Louis, 1984); several non-human primates with a variety of different *Leishmania* species (Lainson & Bray, 1966; Lainson & Shaw, 1977; Christensen & Vasquez, 1981; Walton, Harper & Neal, 1983; Dennis, Chapman, Hanson & Lujan, 1985; Dennis, Lujan, Chapman & Hanson, 1986; Lujan, Dennis, Chapman & Hanson, 1986; Githure, Reid, Benhazim, Anjili, Shatry &

Hendricks, 1987; Peireira, Melo, Mayrink & De Resende, 1988). Some of these studies are pertinent to vaccine development but will not be considered here. The bulk of information on immunology and pathology of leishmaniasis has been obtained from mouse models. This is because of the availability of cell markers, inbred, congenic and now even transgenic mice, in addition to all other attributes which have made the mouse the animal of choice for laboratory experimentation.

The mouse

Several laboratories discovered independently that the susceptibility of inbred mice to a given *Leishmania* species (infection caused by promastigotes) varies considerably and is controlled genetically (Kellina, 1973; Handman, Ceredig & Mitchell, 1979; Behin, Mauel & Sordat, 1979; Nasseri & Modabber, 1979; Perez, Labrador & Torrealba, 1979; Howard, Hale & Liew, 1980*a*). Most inbred mice are resistant to *L. major* infection (recover spontaneously) and only a few, notably BALB/c and its H-2 congenic strains are highly susceptible. These mice exhibit visceral disease and invariably die of a fulminating disseminated infection, even with injections of a very small inoculum (e.g. 100 organisms) compared to the 10^6 organisms required to give a small self-healing lesion in resistant mouse strains. This exquisite susceptibility has fascinated many investigators who have used the BALB/c–*L. major* model to study the immunology and also the chemotherapy of leishmaniasis. Unfortunately, *L. major* infection in BALB/c mice cannot be considered a model for any of the human leishmaniases.

The visceralization and the associated signs (changes in serum proteins, peripheral blood cells, anergy to skin test antigens, splenohepatomegaly, etc.) resemble human kala azar (Djoko-Tamnou, Leclerc, Modabber & Chedid, 1981). However, a large lesion is initially produced at the site of inoculation and metastatic lesions develop subsequently quite unlike the human disease. The philosophy of using BALB/c mice for vaccine or drug studies is that if BALB/c can be protected or cured then so can the resistant mouse strains which produce a benign self-healing disease similar to human zoonotic cutaneous leishmaniasis (ZCL). The danger is that some otherwise potentially useful reagents (drugs or vaccines) may be undetected because of the stringencies and peculiarities associated with the BALB/c–*L. major* model.

Influenced by epidemiological impressions, resistance to leishmaniasis has been attributed to cell-mediated immunity as measured by the classical DTH reaction in humans, using leishmanin (phenol-killed *Leishmania*) in a skin test (Montenegro reaction). Early experiments with T cell deprived (irradiated, thymectomized and bone-marrow reconstituted) mice showed the involvement of T cells in the pattern of the disease (Preston, Carter, Leuchars, Davies & Dumonde, 1972). Mitchell *et al.* (1980) clearly demonstrated the role that T cells play in immunity. The lack of DTH in infected BALB/c mice (Nasseri & Modabber, 1979; Howard, Hale & Liew, 1980*b*) and transfer of resistance together with DTH, by use of T cells from protected mice (reviewed by Mitchell, 1984), further pointed to a correlative, if not a causal relationship between DTH and resistance. In fact, in the era of dominance of suppressor T cells in immunological phenomena, the susceptibility of BALB/c mice was thought to be due to the induction of suppressor T cells which specifically down-regulated DTH response (Liew, Hale & Howard, 1982). The first indication that there was not a causal relation between DTH and resistance was obtained from studies on F_1X backcross of A/J × BALB/c mice infected with *L. major* (Modabber, Alimohammadian, Khamesipour, Pourmand, Kamali & Nasseri, 1980). The time of death after infection did not correlate with the presence or magnitude of the DTH response. The elegant works of Louis and his collaborators (Lima, Engers & Louis, 1984; Milon, Titus, Cerottini, Marchal & Louis, 1986) and Liew, Howard & Hale, (1984) clearly showed that DTH is not the protective mechanism *per se*.

In the first successful immunization using killed organisms, Howard, Nicklin, Hale & Liew (1982) showed that intraperitoneal (i.p.) or intravenous (i.v.) immunization with killed *Leishmania* rendered BALB/c mice immune to *L. major* infection, yet the mice at the time of challenge were DTH-negative. The classical observation by Titus, Lima, Engers & Louis (1984) that *Leishmania*-specific T cell lines which can activate infected macrophages to kill the parasite *in vitro* will exacerbate the disease when transferred *in vivo*, was surprising but crucial in the understanding of the mechanism of the host–parasite relationship. Not only did it become clear that *in vitro* observations could not simply be extrapolated to *in vivo* situations, but these findings showed that activation of macrophages to kill intracellular parasites *per se* was not sufficient to control the disease. Other factors, such as (*a*) different cytokines or (*b*) parasite products, might override the crucial effector mechanism, macrophage activation. Evidence for both mechanisms exists: (*a*), firstly infected BALB/c mice produce an inexplicably high concentration of colony stimulating factors (CSF) and a high number of granulocyte–macrophage colony forming cells in culture (GM CFU-c, immature monocytes) (Mirkovich *et al.* 1986). These premature cells act as 'safe targets' for the parasite, presumably because they cannot be activated to kill the parasites (Hoover & Nacy, 1984). Titus *et al.* (1984) noted that exacerbating T cells induced accumulation of macrophages at the site of the lesion, and also suggested that this

may be a mechanism by which the disease is exacerbated. In support of the 'safe target' hypothesis are the observations that administration of recombinant CSF (Solbach, Greil & Rollinghoff, 1987) or IL-3, multi-colony stimulating factor acting on bone-marrow precursor cells (Feng, Louis, Kindler, Pedrazzini, Eliason, Behin & Vassalli, 1988), will aggravate the disease in mice.

Alternatively, these lymphokines may directly affect the mediators of macrophage activation, such as gamma-interferon (IFN), as suggested by the more recent results of Liew *et al.* (submitted for publication). These investigators have presented evidence indicating that the 'exacerbating' T cells produce factors which can inhibit the IFN-mediated activation of macrophages and thereby prevent intracellular killing of *Leishmania*. The factors involved are identified as interleukin 3 (IL-3) and IL-4 (B cell stimulating factor also affects mast cell differentiation). This observation is crucial for explaining exacerbation of the disease in the presence of large numbers of effector T cells in BALB/c mice and the possible mechanism by which the unbalanced T cell populations would lead to modification of the immune responses (reviewed by Muller *et al.* 1989).

Recently, Kaufmann & Flesch (1988) have shown that IL-4 and IL-5 (eosinophil differentiation factor) potentiate the IFN-mediated activation of macrophages to kill *Mycobacteria* and other intracellular organisms. This is an apparent contradiction with the observations of Liew *et al.* (submitted). The difference in the experimental design and the state of 'activation' required for killing bacteria versus *Leishmania* may account for this apparent contradiction. The subdivision of T-helper (L3T4+) cells into the two groups TH1 and TH2 on the basis of lymphokine production (Mosmann & Coffman, 1987), provides an explanation for the dual and contradictory roles described for L3T4+ *Leishmania*-specific T cells, mediating resistance as well as abrogation of resistance (Liew *et al.* 1982; Titus, Milon, Marshal, Vassali, Cerottini & Louis, 1987). Disregarding some overlap between the two cell types, TH1 and TH2 cells are distinguished by production of IFN and IL-4, respectively. It follows then that TH1 cells would be expected to protect and TH2 cells to exacerbate. Indeed, Locksley, Heinzel, Sadick, Holaday & Gardner (1987) have shown that resistance in mice correlates with the ability to produce TH1-type cells.

Secondly, (*b*), parasite products may directly interfere with the mechanism of activation of macrophages (Handman, Schnur, Spithill & Mitchell, 1986). It has been shown by Flesch & Kaufmann (personal communication) that products of *M. leprae* can suppress the IFN-mediated activation of bone-marrow derived mononuclear phagocytes *in vitro*.

It is not known what factors influence the induction of different subpopulations of cells. Operationally, the route of injection and the use of adjuvants determine the predominant cell type induced, for example, subcutaneous injection of leishmanial antigens with Freund's complete adjuvant will lead to preferential induction of exacerbating cells (TH2), while i.v. or i.p. injections of the parasite lead to predominant generation of protective T cells (TH1). This may appear to be a major problem in the development of human vaccines. However, as mentioned above, numerous studies in humans indicate that exposure to the leishmanial antigens via the cutaneous route does not sensitize the individual to produce an exacerbated disease when subsequently infected with *Leishmania* (Mayrink *et al.* 1985; Antunes *et al.* 1986; Nadim, personal communication). More recently, Russell & Alexander (1988) have shown that leishmanial antigens, particularly the promastigote surface protease (gp63) and the major surface glycoconjugate of Handman, Greenblatt & Goding (1984), a component of the excretory factor described by Schnur, Zuckerman & Greenblatt (1972), could induce a protective immune response when incorporated in liposomes. This immunity was shown to be transferable by T cells. Hence presentation of antigen can profoundly affect the ensuing immune responses.

The specificity of the protective cells (presumably TH1) and the exacerbating L3T4+ cells (presumably TH2) are not known. Is there a repertoire restriction of epitope specificity? This question is obviously very important in developing molecularly defined vaccines. If the specificity of TH1 cells is limited to certain antigenic moieties, then the choice of molecules would be of crucial importance. On the other hand, if there is no restriction with respect to epitope specificity for TH1 and TH2, then a large number of immunogenic molecules could be used effectively, provided that they are presented in a form (right vehicle, adjuvant, carrier and route of injection) that can preferentially or exclusively induce a TH1 response. Data to support both arguments exist. According to Mitchell & Handman (1986) the carbohydrate part of the LPG, is a 'suppressogenic' molecule – an unfortunate term to denote a 'parasite-protective' molecule (a better term used by the same authors) – e.g. a TH2-stimulating immunogen, whereas the whole molecule (LPG) would be a TH1-inducing and hence a protective immunogen.

The mechanism of exacerbation produced by the carbohydrate alone is not known, nor is the specificity of T cells produced after carbohydrate injection. Do T cells recognize the carbohydrate, or is the exacerbation mediated by B cells and their products? In other words, does the carbohydrate moiety induce a TH2 response directly or via B cells? It is known that carbohydrate immunogens only stimulate antibody formation and not DTH.

The protective effect of anti-μ treatment of BALB/c (Sacks, Scott, Asofsky & Sher, 1984) is compatible with a B cell-mediated susceptibility.

A more attractive hypothesis is that the nature of the response is regulated by the antigen-presenting cells (APC). In this model, the repertoire of T cell specificity is not so limited as to exclude certain groups of epitopes (including carbohydrate epitopes). Hence, if the epitope is presented properly and all the required stimuli are available, the specific T cell clone will be stimulated. The observation of Russell & Alexander (1988) with gp63 in liposomes, supports this notion. If so, then selection of molecularly defined potential vaccines would be easier and would be based on their presence on those APC which best induce a TH1 equivalent response in humans. Several cell types are known to be APC, e.g. macrophages, dendritic cells and B cells. Of these, it is expected that B cells would not be the APC of choice for a protective response against leishmaniasis, for the reasons mentioned above. The analysis of antigens presented by APC (other than B cells) would be crucial in the selection of molecularly defined vaccines.

One of the most important achievements of recent years in the study of murine leishmaniasis is the generation of T cell lines and clones which can protect BALB/c mice against an otherwise lethal challenge of *L. major*. This has been accomplished in two laboratories independently. Muller & Louis (submitted for publication, reviewed by Muller *et al.* 1989) have established two protective cell lines which recognize only antigens associated with the live *Leishmania*. These cells were generated in the presence of live *Leishmania*, in contrast to the previously generated T-cell lines and clones which were all exacerbating and for which parasite extracts were used. These authors believe that to be protective, T cells must recognize an antigen associated with the live parasite, since the T cells must be able to activate infected macrophages (containing live parasites) in order to be protective. Otherwise T cells which can only recognize antigens on the surface of the macrophages which have ingested dead parasites, could not be protective even if they are of the TH1 type. This model argues very strongly for the importance of epitope specificity and imposes a new requirement for TH1 cells in order to be protective. Use of the TH1 versus TH2 model to explain the diversity of functions by cells of the same phenotype would still remain valid if macrophages which have ingested dead parasites would also present some antigens presented by the infected macrophages and vice versa. It follows that only a proportion of TH1 cells would be involved in elimination of the infection. This proportion depends on the number of common antigens present on the surface of macrophages which have ingested dead organisms and the ones infected with the live parasites. The analysis of antigens present on these two types of macrophages is important.

Scott, Natovitz, Coffman, Pearce & Sher (1988) have used a protective fraction of the parasite, which they had identified previously (Scott *et al.* 1987), to generate their protective line from which they have produced a protective clone. These cells, in contrast to those produced by Muller & Louis (1989), mentioned above, will recognize the protective fraction without requiring live parasites. It is not known whether the antigen recognized is present on the surface of infected macrophages, a feature which would support Muller & Louis's model.

The protective fraction of Scott *et al.* (1988) may contain the antigen or be the one that is also presented on the surface of *infected* macrophages. On the other hand, the specificity of the protective clones of Muller and Louis may have been achieved totally by chance and it may be that if many more clones are produced, antigens common to infected macrophages and macrophages that have ingested dead parasites, or a fraction thereof, would be found. Nevertheless, the specificity and functional analysis of these and future protective cells will be needed to understand the mechanism of immunity.

The requirements for an immunogen to be protective, extrapolated from the mouse studies as they are known today, seem to be the following. First, the immunogen must be presented in such a way that it would not produce a local inflammatory reaction (see Titus & Ribero, 1988). Second, the immunogen must be introduced so that it will be presented by the appropriate APC which can induce the desired immune responses (see Russell & Alexander, 1987). Third, the immunogen must induce predominantly, if not exclusively, a TH1 type T cell response, i.e. IFN-producing in the mouse (see Locksley *et al.* 1987), or their equivalent in the human, if they exist. These cells must recognize an epitope on the surface of infected macrophages, since activation of the infected macrophage is the necessary mechanism required for killing of the intracellular parasites (see Muller *et al.* 1989). Fourth, the immunogen must not produce a strong T or B cell stimulation (see Mitchell, Handman, Moll, McConville, Spithall, Kidane, Samaras & Elhay (1987) and Milon *et al.* (1986) on enhanced T cell response, and Colle *et al.* (1983) and Lohoff, Matzner & Rollingholl (1988) on polyclonal B cell response).

With the information available, the ease with which *L. major* can be cultured, the relatively benign nature of cutaneous leishmaniasis and the possibility of testing candidate immunogens in man, it is not surprising that some of us 'Leishmaniacs' are very optimistic and believe that if BALB/c mice can be protected now, humans will be protected in the not too distant future.

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