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Part II.III *Leishmania* Antigen Presentation and T Cells

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The method by which antigen is presented to the T-cell domain by antigen presenting cells is obviously critical to the T-cell response where T-cells of the same phenotype are capable of expressing both helper and suppressor functions. There is evidence that the configuration of the antigenic molecule on presentation can alter the T-cell response.

In our hands the only effective antigen presenting cell *in vitro* is the macrophage although spleen cells have been effective with others. While whole soluble antigen is an effective inducer of T-cell proliferation, the fractions coming off first from a Sephadex 100 column contain most of the effective stimulatory antigens. Following infection in CBA/Ca mice the antigen still present in lymph node cells is sufficient to initiate primary T-cell proliferation. In our hands colonies of T-cells can be transferred and kept alive for several weeks but colonies do not continue to proliferate beyond the initial growth despite the presence of interleukin II.

Part III. *Leishmania* Biochemistry and Chemotherapy

Chaired by Dr D L Goodwin

Part III.I Antileishmanial Chemotherapy Reviewed

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Introduction

The early development of chemotherapy was stimulated by the realization that some dyes such as methylene blue were able preferentially to stain particular cells and this idea developed into the concept of chemical substances which could unite with and inhibit parasitic agents of disease – the so-called magic bullet theory of Paul Ehrlich. There was next a quantum leap to the use of arsenic compounds against African trypanosomes, particularly a supposedly less toxic compound atoxyl, originally prepared by Bechamp in 1863 and used clinically at that time for treatment of skin conditions and anaemia.

A smaller conceptual leap in thinking was then to the use of antimony compounds such as tartar emetic which like the arsenic compounds was also trypanocidal. The first use of antimonials in leishmaniasis in man is credited to Vianni who used tartar emetic in Brazil to treat a patient with cutaneous leishmaniasis. Since that time other antimonial drugs have been studied such as urea stibamine, sodium antimony tartrate, stibophen, anthiomaline, stibamine, neostibophen, neostamine, sodium stibogluconate, meglumine antimoniate and many others.

From about 1950 it became apparent that the pentavalent compounds were much less toxic clinically than the trivalent antimonials, and today treatment is confined primarily to two pentavalent antimonials, sodium stibogluconate and meglumine antimoniate. The organo derivatives of oxo-antimony anions are ill-defined and exhibit various degrees of polymerisation which makes complete chemical quality control of the products impracticable. However, modern antimonial drugs, with standardization of conditions of synthesis, chemical analysis of pentavalent antimony combined with biological control of toxicity and efficacy, give the best possible standard compound. Sodium stibogluconate in aqueous solution has a mean molecular weight of about 400.

The Clinical Situation

From the clinical point of view, the drugs currently available for treatment of all forms of leishmaniasis are the two pentavalent antimonial compounds, sodium stibogluconate and meglumine antimoniate, the polyene antibiotic amphotericin B used primarily as second line treatment for Latin-American cutaneous leishmaniasis

and the synthetic diamidine pentamidine used mainly for second line treatment of visceral leishmaniasis. The drawbacks associated with these drugs are the parental administration of the antimonials and the toxic side effects of amphotericin B and pentamidine. All drugs are given in multidose schedules.

Recently, clinical studies on the pharmacokinetics of sodium stibogluconate have shown that, contrary to earlier belief, the drug is excreted very quickly. Work in Nairobi showed that after a dose of 10mg Sb/kg, serum levels peaked briefly at about 10mg Sb/litre. More than 90% of the antimony had been excreted within six hours. There was little evidence of accumulation in man (Rees *et al.*, 1980).

A multidose study by the Walter Reed research group in Washington also showed a rapid excretion of antimony with a gradual rise of preinjection antimony concentration in the whole blood sample. The rise was more marked after intramuscular injection of meglumine antimoniate than intravenous sodium stibogluconate. Both drugs were given at a dose level of 10mg Sb/kg/day. The data were best fitted to a three compartment kinetic model with half-lives of each compartment of 0.34, 1.72 and 32.8 hours (Pamplin *et al.*, 1981; Pamplin, unpublished observations).

These studies show that, with a single injection every 24 hours as recommended at present, during most of this time the blood or serum concentration is below the level required to inhibit the amastigotes. Clearly the study of more frequent administration of pentavalent antimonials is necessary. However, tissue concentrations, particularly intracellular concentrations, are the most relevant and this information is not available at present. With the rational development of more effective schedules of treatment, it was hoped that kala-azar would become better controlled and the proportion of cases unresponsive to therapy would diminish. In fact these predictions are being fulfilled.

Studies with Indian kala-azar patients by Thakur and his colleagues (1984a, b) have shown that the standard dose of sodium stibogluconate of 10mg Sb/kg can be given for 20 or more injections and this increased number of injections resulted in a reduction in the proportion of non-responding patients from 15 to 0.5%. Studies in Kenya were similar and also included raising the dose to 15mg Sb/kg with a similar improvement in efficacy (Chulau *et al.*, 1983; Rees *et al.*, 1984; Chunge *et al.*, 1984). ECG studies in kala-azar patients in Kenya have shown that treatment with very high dose levels of sodium stibogluconate can produce side effects (Chulau *et al.*, 1985). As a result of their extended studies, it was concluded that for patients receiving 10mg Sb/kg/day for up to 30 days or 20mg Sb/kg/day for up to 20 days, no ECG monitoring was required. However, patients receiving 20mg Sb/kg/day for more than 20 days, or a higher dose for more than 10 days, should have ECGs every 3–4 days. At the highest dose schedules, life threatening arrhythmias may develop.

These new investigations show that increasing the dose of pentavalent antimony as sodium stibogluconate in the treatment of kala-azar, results in a dramatic reduction of the number of unresponsive patients previously known as “drug resistant” patients. However, there is clearly a limit to the amount of drug which can be given, depending on individual circumstances.

Treatment of cutaneous disease is a more difficult problem than that of visceral infection. With regard to the Old World cutaneous leishmaniasis due to *L. major*, there is no generally accepted treatment and no properly controlled clinical trial has yet been published. This is probably because the disease is not life threatening, not usually mutilating and is self limiting. It is however often painful and disfiguring. In view of the anti-*Leishmania* properties of the pentavalent antimonial compounds, a clinical study would be of great interest. From available information, pentavalent antimonials may be effective against *L. major* and *L. tropica* infections in multiple courses, while *L. aethiopica* is less susceptible than *L. major*.

The treatment of *L. braziliensis* infection in Latin-America has been studied in some detail and again the pentavalent antimonials are the current drugs of choice. The drug used is meglumine antimoniate since sodium stibogluconate is not available in Latin-America. Since meglumine antimoniate is recommended by the manufacturer to be given at higher dosages than sodium stibogluconate, the necessity for increasing the antimony dosage is not so immediate. The schedule generally recommended is 50–60mg/kg (= 17–20mg Sb/kg) for 12 days. However, studies by Marsden and colleagues (Marsden *et al.*, 1985; Sampaio *et al.*, 1985) have shown that increasing the dose schedule can result in cure in patients previously known to be unresponsive to treatment.

For second line treatment of difficult cases such as those with mucosal involvement, amphotericin B is generally used in Latin-America, although for Indian post-kala-azar dermal leishmaniasis (PKDL), pentamidine is used.

The greater difficulty of treatment of cutaneous leishmaniasis may be due to two main factors; the drug does not reach the parasite in the skin, or the parasite may have a lower susceptibility to the drug. In the case of *L. major*, recent studies with amastigote infected macrophages *in vitro* have shown that the ED₅₀ for sodium stibogluconate ranges from 4 to 10 times higher than that of *L. donovani*. On the other hand, *in vitro* studies with one isolate of *L. braziliensis braziliensis* and with isolates from PKDL, showed they were as sensitive as *L. donovani* (Neal, unpublished observations).

In experimental mouse infections with *L. donovani*, the parasite load is easily controlled with sodium stibogluconate, giving a calculated ED₅₀ of 10 to 15mg Sb/kg/day × 5, while cutaneous infections with *L. major* cannot be eliminated with the drug. Lesions of mice

were controlled by the very highest levels administered, and an ED₅₀ was calculated to be 305mg Sb/kg/day × 10. This new evidence shows that amastigotes of *L. tropica* and *L. major* have a lower susceptibility *in vitro* to antimonials, but adverse tissue distribution contributes to the problem.

Acquired Drug Resistance in Leishmaniasis

As mentioned above, failure to respond to treatment was not uncommon with kala-azar and was often attributed to drug resistance. However, since increased dose levels now cure most patients, the problem was more likely to have been inadequate treatment.

True drug resistance has been reported by Hanson and his colleagues (1982) who observed that a Kenyan strain of *L. donovani* from a non-responding patient was relatively insensitive to meglumine antimonate in infected hamsters. The resistant strain was also less sensitive *in vitro* in amastigote infected macrophages (Berman *et al.*, 1982). The latter workers also found other leishmanial isolates which were less susceptible *in vitro*. These latter strains were isolated from patients who contracted their cutaneous lesions in Panama and required more than one course of treatment. The exact identification of the parasites used was not always given, but most were *L. b. panamensis*.

Thus, it is now clear that antimony resistance is a real entity, although further studies are necessary to evaluate if it is a clinical problem. So far I have analysed *L. major* isolated from five patients not responding to treatment with antimonial drugs, and lack of susceptibility was not confirmed in any of these cases. Further isolates are required, especially from other leishmaniasis.

Guidelines for drug trials

Before leaving the clinical situation I would like to draw your attention to the drug trial protocols published by the World Health Organisation. These resulted from three meetings of groups of people working in these fields of research, and the protocols are available for Latin-American muco-cutaneous leishmaniasis (TDR/LEISH/MCL/79.3), visceral leishmaniasis (TDR/CHEMLEISH/VL/82.3) and Old World cutaneous leishmaniasis (TDR/LEISH/CL-JER/82.3).

New Therapeutic Developments

There are three areas of possible development.

1. Local treatment of cutaneous lesions

One of the most active drugs against *L. major* is the aminoglycoside, paromomycin (Neal, 1968). This has been used clinically by Soviet workers under the name monomycin. Dr El-On and his colleagues (1984) in Jerusalem found that by combining paromomycin with a quaternary ammonium compound, particularly methylbenzethonium chloride, the transdermal penetration of paromomycin was improved and when applied to mouse lesions due to *L. major* for up to 10

days, effectively promoted complete and rapid healing. However, not all amastigotes were eradicated since lesions reappeared 90 to 150 days after the end of treatment. The mouse strain used for this work was BALB/c and is known to be highly susceptible to *Leishmania* infections. Since man mounts an efficient immune response, it is possible that relapse may not occur in clinical use. Results of controlled clinical trials have not yet been published, although the patent reports on the successful treatment of a small number of patients (UK Patent No. 8209231).

Similar studies with *L. b. panamensis* infected *Myristomys albicaudatus* showed that application of an ointment containing sodium stibogluconate for one month, resulted in the healing of the lesion, but again not all amastigotes were eliminated since the lesions subsequently reappeared (McGreevy *et al.*, 1981).

Other drugs with known antileishmanial properties are being considered for topical treatment. These include the antifungal agents clotrimazole, miconazole and ketoconazole, and the tranquillizer, chlorpromazine. So far only individual cases have been treated either systematically or topically and further studies are required to establish the value of these drugs.

2. Liposome encapsulated drugs

After intravenous injection, liposomes with entrapped drug are taken up by the phagocytic cells of the liver and spleen. Since these cells also contain the amastigotes, it is clear that the drug would inhibit the parasites without affecting the function of other organs of the host. Data on liposome entrapped sodium stibogluconate treatment of *L. donovani* infections in mice and hamsters indicate a much greater activity compared to free drug. Black *et al.* (1977) reported more than 200-fold, whilst New *et al.* (1978) also found high activity. Entrapped meglumine antimonate was also highly active (Alving *et al.*, 1978). Liposome entrapped drug was less effective against experimental cutaneous leishmaniasis (New & Chance, 1980; New *et al.*, 1981; Panosian *et al.*, 1984).

More recently Chapman *et al.* (1984) have shown that dogs experimentally infected with *L. donovani* were very susceptible to liposome treatment, the 50% suppressive dose being reduced from 25mg Sb^v/kg/day × 4 for free meglumine antimonate to 0.029mg Sb^v/kg/day × 4 for liposome encapsulated drug. The liposomes were estimated to be more than 700 fold more active than the free drug. This is similar to the earlier results from infected hamsters.

Unlike some earlier liposome preparations, a method has been developed by Rao (1983) for the production of stable, non-leaking liposomes. Perhaps of even greater interest is the observation of Rao and colleagues (1983) using whole body gamma scintigraphy, that liposome encapsulated sodium stibogluconate remains localised in the liver and spleen for prolonged periods. Some radioactivity was still visible after 6 days. Clearly, these

observations give promise of a reduction of the number of injections of liposome encapsulated drug material.

3. New Chemical series

There are two new drugs awaiting completion of development. The first, allopurinol ribonucleoside, developed by the Wellcome Foundation Ltd, is currently in a small scale clinical trial (phase 2) against cutaneous leishmaniasis in Panama (Dr Hutchinson, personal communication). This compound was selected from the pyrazolopyrimidine series. It has a very low toxicity to man and animal, but although it has antileishmanial properties (see review by Marr, 1984) its activity in laboratory models is not high (Neal *et al.*, 1985) Final results of the clinical trials are awaited with interest.

The second drug is a 4-methyl-8-aminoquinoline, known by the number WR-6026 (Kinnamon *et al.*, 1978). This compound was discovered during empirical studies of 8-aminoquinoline compounds synthesised for antimalarial activity at the Walter Reed Army Institute for Research, and is currently awaiting phase 2 clinical studies.

At the level of innovative research, there are a number of leads which may or may not develop further. The most promising perhaps are the antifungal imidazole and triazole derivatives. Others include phenothiazine tranquilizers, tricyclic antidepressants and sinefungin analogues of s-adenosyl-methionine.

Outlook

Clearly there has been very little progress since the beginning of the era of chemotherapy. Treatment of disease is usually satisfactory with pentavalent antimonials with a cumbersome dose schedule which only now, 50 years or more after their discovery, has been put on a rational basis. The reasons for this failure are many and varied, but basically are due to too few resources being applied to the problems. However, improvements in therapy could be imminent if only the speed of development of new products could be increased.

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