Successful Use of a Defined Antigen/GM-CSF Adjuvant Vaccine to Treat Mucosal Leishmaniasis Refractory to Antimony: A Case Report

Roberto Badaro, Iza Lobo, Maria Nakatani, Alvaro Muiños, Eduardo M. Netto, Rhea N. Coler and Steven G. Reed

Federal University of Bahia, Salvador, BA , Brazil; Corixa Corporation Seattle, Washington, USA

Immunotherapy has been proposed as a method to treat mucosal leishmaniasis for many years, but the approach has been hampered by poor definition and variability of antigens used, and results have been inconclusive. We report here a case of antimonial-refractory mucosal leishmaniasis in a 45 year old male who was treated with three single injections (one per month) with a cocktail of four *Leishmania* recombinant antigens selected after documented hypo-responsiveness of the patient to these antigens, plus 50mg of GM-CSF as vaccine adjuvant. Three months after treatment, all lesions had resolved completely and the patient remains without relapse after two years. Side effects of the treatment included only moderate erythema and induration at the injection site after the second and third injections. We conclude that carefully selected microbial antigens and cytokine adjuvant can be successful as immunotherapy for patients with antimonial-refractory mucosal leishmaniasis.

Key Words: Leishmaniasis, immunotherapy, mucosal leishmaniasis.

Mucosal leishmaniasis (ML) is a severe disfiguring disease that usually evolves chronically and is extremely difficult to treat [1]. ML or mucocutaneous leishmaniasis (MCL) presents a very different clinical, pathological and therapeutic problem than does cutaneous leishmanial infection. Once leishmanial granulomas are present in the mucosa, pentavalent antimonials often fail to cure the disease [2, 3]. Several attempts to treat MCL patients heavily exposed to antimonial therapy have been reported [4]. However, none of the alternatives have shown sufficient efficacy to recommend them as the solution for treatment of refractory MCL cases [5]. In contrast, reports of the efficacy of immunotherapy with crude *Leishmania* antigen preparations, in combination with BCG (Bacillus Calmet-Guerin), have indicated that there could be dramatic healing responses of the lesions in patients with CL and MCL by use of antigen vaccines [6-9]. Such vaccines are accepted in South America as alternative, non-standard, and perhaps promising, but seldom used, approaches to the treatment of leishmaniasis [5].

Here, we present a case report of a patient in whom immunotherapy with specific recombinant leishmania antigens, in combination with a potent cytokine as vaccine adjuvant, was used successfully to treat active mucocutaneous leishmaniasis.

Case report

The patient was a 45-year-old male agricultural engineer with a history of ulceration of the right foot and severe mucocutaneous leishmaniasis (ML) that was first noted six years prior to evaluation for treatment with immunotherapy. Four months after the appearance of an ulcer on his foot, a diagnosis of cutaneous leishmaniasis (CL) was made at the health post in his hometown in Bahia, Brazil, by identification of *Leishmania* organisms in a biopsy of the lesion, and a

Received on 12 July 2001; revised 15 August 2001.
Address for correspondence: Dr. Roberto Badaró. Hospital Universitário Professor Edgard Santos, Unidade Docente Assistencial de Infectologia 6nd. Rua João das Botas, s/n Zip code: 40160-110. Salvador – Bahia –Brazil.
E-mail: badaro@ufba.br

The Brazilian Journal of Infectious Diseases 2001;5(4):223-232

1413-8670

positive Montenegro skin test. At that time, he was treated with pentavalent antimony (Sb^v) 850 mg daily for 20 days. After two consecutive courses of 20 days of antimony therapy, the ulcer was completely healed. Three months later, the patient noted a small lesion in the nasal septum, with swelling of the nostril. He was retreated with antimony using the same dose schedule. After 40 consecutive injections of Sb^v, the lesions in his nose were still active. The local physician referred him to the Infectious Diseases Unit at the University Hospital Professor Edgard Santos, Salvador, Bahia, Brazil. The diagnosis of active MCL was confirmed. He was treated in hospital with the alternative drug of choice, Amphotericin B. After a total dose of 500 mg, he developed severe Amphotericin B adverse reaction (- BUN and creatinine, fever and arrhythmia). Three weeks after the discontinuation of Amphotericin B, all adverse reactions disappeared. However, the mucosal lesions, despite some improvement, were still active. The patient refused further treatment with Amphotericin. A third round of drug therapy was tried with a combination of Sb^v and Aminosidine. After 20 days of treatment, a slight improvement was noted. The patient developed severe arthralgia. Another course of therapy was scheduled for three weeks later, however, he did not return to the hospital for four months, at which time he had complete relapse of the MCL characterized by total destruction of the nasal septum, and redness and swelling of the lips. At that time, his physicians proposed treatment with a combination of immunotherapy using a preparation of heat killed Leishvacin® (*Leishmania mexicana amazonensis*) (prepared by Biobrás/Brazil) with pentavalent antimony following the successful anecdotal trial reported by Mayrink, *et al*. [7].

After the tenth dose of the Leishvacin® in combination with Sb^v, no response was noted. The patient was very frustrated and asked for another alternative treatment. A combination therapy of Pentamidine 4 mg/Kg/daily, three doses per week, plus standard daily doses of Sb^v were initiated. After 12 injections of Pentamidine, a dramatic response was noted with healing of most the lesions in the nose. This successful therapeutic combination could not be continued, however, because the patient developed a severe adverse reaction to Pentamidine. He developed insulin dependent diabetes.

Six months later, the mucosal lesions completely relapsed again. He returned to the hospital and was retreated with a fourth alternative drug combination using Alopurinol 300mg daily plus Antimony (850 mg/day). After three courses of 20 days of this combination therapy, a slight improvement was noted. Again, the patient developed severe arthralgia and his nose started to bleed more frequently. He decided to abandon the treatment and return home. Four months later, he returned to the hospital with a complete relapse of the mucosal disease with facial disfigurement and total destruction of the nasal septum. Figures 1A and 1B show his face before immunotherapy. By this time, he was suffering from depression, nearly to the point of suicide, and begged us to find a solution for his disease.

Because there was no other chemotherapy to offer him, he was informed of promising laboratory results following the use of several new recombinant proteins (isolated by Corixa Corporation, Seattle, Washington) that had been shown in animal experiments to be extremely potent Th1 type cytokine response inducers. The patient volunteered for this experimental treatment making the following statement: "*…it is much better to try anything, even if it can kill me, than live with my face like it is."* We evaluated his *in vitro* T-cell proliferative responses to various available antigens in order to prepare a vaccine cocktail for the immunotherapy. (See Methods Section)

The patient was injected subcutaneously with the prepared recombinant vaccine. During the next 12 hours, he was kept in the hospital for observation of any adverse reaction. Thirty days following the first injection, he returned to the hospital very happy because the inflammation in his nose started to ameliorate and he had no more episodes of bleeding from the nose. A second dose of the vaccine was injected (one month after the first) in the same way as the first injection. Thirty days after the second dose, his face was almost completely healed and the granulomatous lesions in the nose were almost gone. He reported that 24 hours after the second dose he experienced a flu-like syndrome and

he noted a skin reaction similar to the Leishmanin skin test at the injection site. Because the lesions were not completely healed it was decided to give one additional dose. Forty-eight hours after the third injection, he came for a follow up evaluation and reported that he had fever (38ºC), and his arm presented a strong skin reaction with an induration of 100mm diameter as shown in Figure 2. The adverse reaction was treated with acetaminophen and a topical corticosteroid, with complete remission of the signs and symptoms.

Thirty-five days later, he came back for follow up and was still completely asymptomatic, with no swelling or alteration of his face (Figures 1C and 1D). Rhinoscopy revealed a complete cicatrisation of the mucosal lesions. Figure 3 shows the rhinoscopy prior to the first vaccine dose (3A) and at follow up after the third dose (3B). Sixty days after the last dose, he was still asymptomatic. The T-cell proliferative response was reassessed. Figure 4 shows the cell proliferative responses to the recombinant antigen components of the vaccine cocktail. At 12 month follow up, the patient complained about having to sneeze and a sensation of obstruction at the posterior nostril. Rhinoscopy revealed scar formation without signs of granulomatous reactions. Mucus and secretions were controlled by use of a locally administered vasoconstrictor and topical moisture cream. At the 18 and 24 month follow up visits, the patient remained without reactivation of mucosal disease. At the present time, five years later, he is still asymptomatic.

Materials and Methods

Antigen and adjuvant preparation, immunization schedule and immune response evaluation

Antigen selection

From a library of recombinant *Leishmania* antigens available at CORIXA Corporation, Seattle, Washington, four recombinant antigens were selected from 15 potential antigens that had induced specific T-cell responses *in vitro* by cells from leishmania infected mice. Eight of these antigens were placed *in vitro* with peripheral blood mononuclear cells (PBMC) from the patient and a T-cell lymphoproliferative assay performed. For analysis of the T-cell reactivity, bulk PBMC (2×20^5 cells/well) were incubated for each individual antigen and controls. Proliferative responses were measured by [^{3}H] thymidine incorporation during 18 hour pulses on day 5 of the assay. Controls for proliferative response included phytohemagglutinin (PHA) and soluble leishmania antigen extract (SLA). Figure 5 shows the blastogenic responses (stimulation index) of each individual antigen. As expected, many of the antigens elicited strong proliferative responses. In contrast, the patient's mononuclear cells did not recognize four antigens that had shown a cytokine profile indicative of excellent Th-1 type cytokine responses when tested *in vitro* in the Murine model. These four antigens included TSA antigen, so named because of its homology with a eukaryotic thiol-specific antioxidant protein with a molecular mass of 22.1 kDa. This recombinant antigen had been identified using sera from mice vaccinated with *Leishmania major* promastigote culture filtrate protein plus *Corynebacterium parvum* to screen a *L. major* amastigote cDNA expression library. Immunization of BALB/c mice with recombinant TSA protein resulted in the development of strong cellular immune responses and conferred protective immunity against infection with *L. major* when the protein was combined with IL-12. In addition, recombinant TSA protein elicited *in vitro* proliferative responses from peripheral blood mononuclear cells of human leishmaniasis patients and significant TSA protein-specific antibody titers were detected in sera of both CL and VL patients [10]. A LmST11 recombinant protein is a *L. major* stress inducible protein that contains six copies of a tetracopeptides consensus motif with a molecular size of 62.1 kDa. This protein was cloned by screening a *L. major* amastigote cDNA expression library with sera from *L.major* infected BALB/c mice. LmSTI1 was shown to elicit strong proliferative responses from draining lymph node cells of *L. major*-infected BALB/c mice at both early (10 days) and late (28 days) stages of infection and to induce production of high levels of IFN-γ and low levels of IL-4. Thus, LmSTI1 is a powerful *Leishmania* antigen capable of eliciting strong T-cell responses, with a Th-1 bias. In addition, analyses of

Figure 1. A/B: These figures show the swelling and complete inflamation of the patient's face due to active leishmaniasis. **C/D:** These figures show the dramatic improvement of the inflamation in the patient's face followed the third dose of immunotherapy

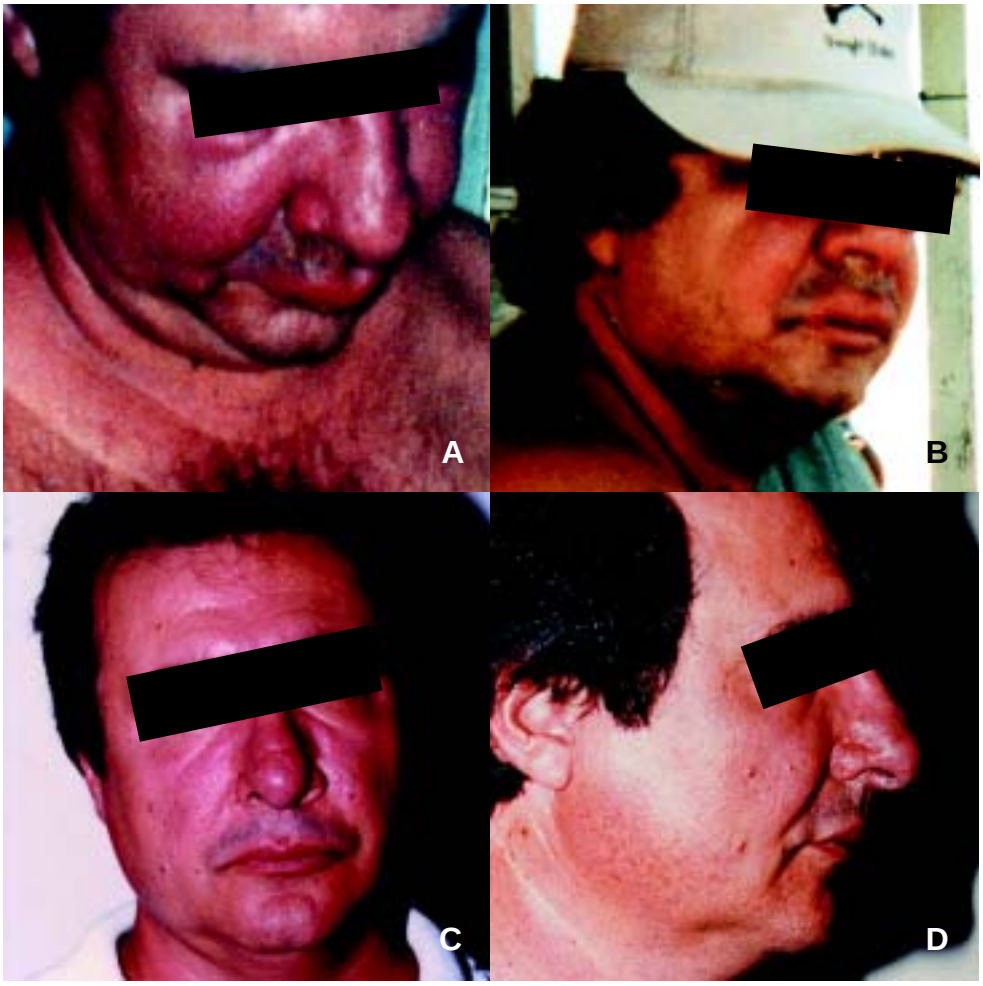


Figure 2. The skin adverse reaction at the site of vaccine injection. The arrow shows the limits of indusation (100mm)

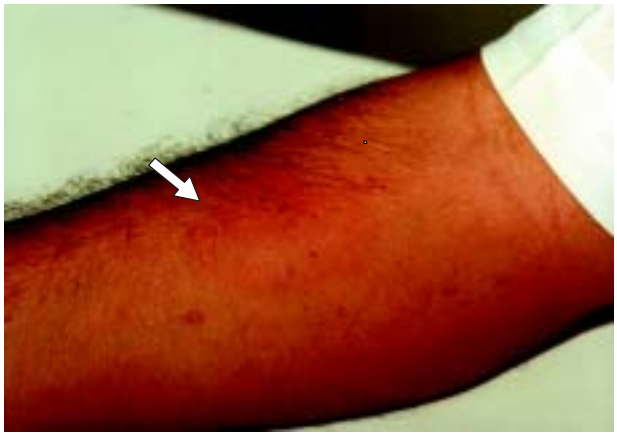


Figure 3. This figure shows the aspect of mucosal lesions in rhinoscopy evaluation pre-therapy (3A), which reveals several mucosal areas with edema and bleeding

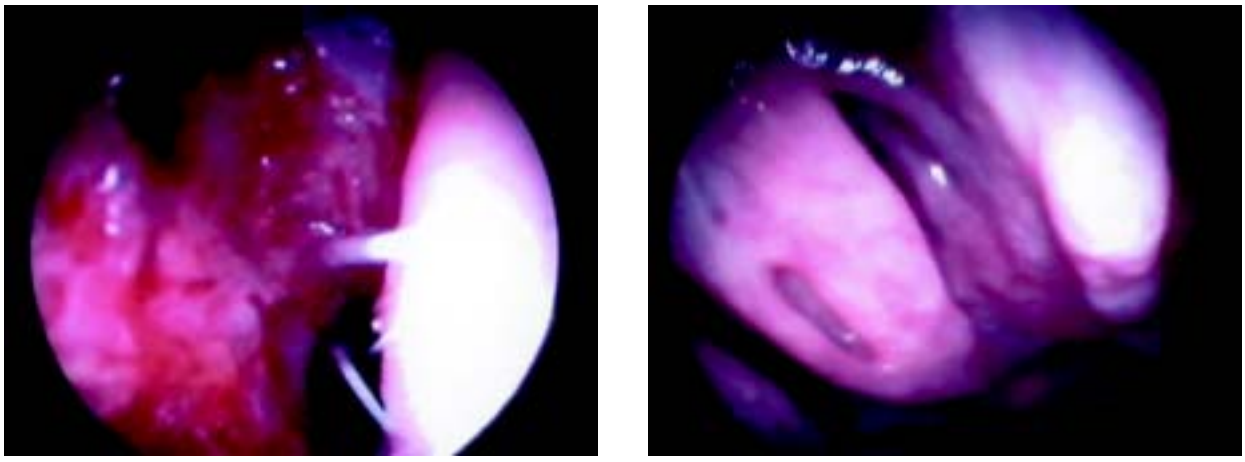


Figure 4. Proliferative response of PBMC from mucosal leishmaniasis patients to recombinant antigens components of the vaccines

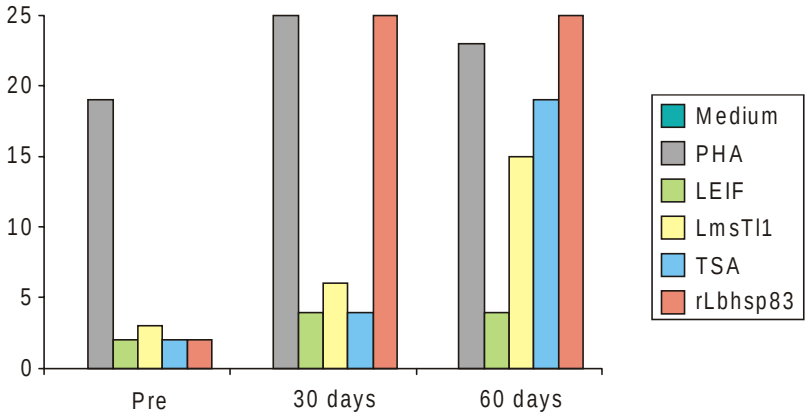
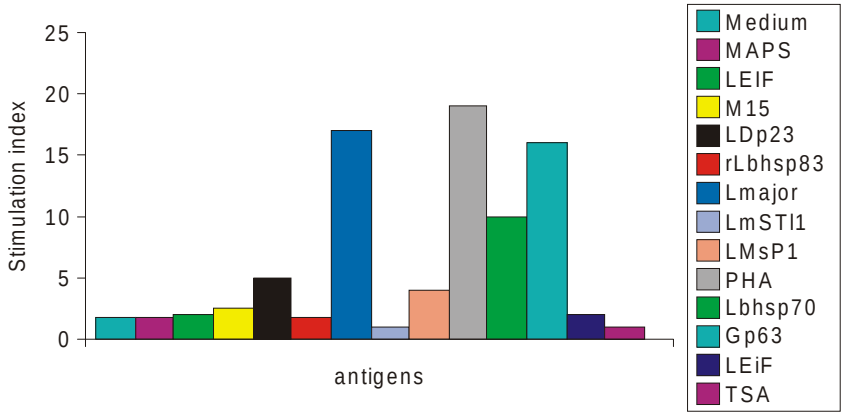


Figure 5. Proliferative response of PBMC from mucosal leishmaniasis patients to recombinant antigens



sera and PBMC from human patients with cutaneous, visceral, and post-*kala azar* visceral leishmaniasis have indicated that a majority of individuals from all three clinical groups mounted strong humoral and T-cell responses against LmSTI1 [11]. A protein termed rLbhsp83 is an 83-heat shock protein of *L. brasiliensis* genes that contains a potent T-cell epitope(s). This protein induces proliferative responses of human peripheral blood mononuclear cells and a mixed Th1-Th2 pattern of cytokine production, depending on the portion of the rLbhsp83 used to stimulate PBMC. The rLbhsp83a portion of the molecule stimulates Interleukin-2 (IL-2), Interferon g (IFNg) and TNFa. The rLbhsp83b portion stimulates IL-4 and IL-10. Its molecular weight is 43kDa [12]. The LeIF antigen expressed from a *L. brasiliensis* gene is homologous to a eukaryotic ribosomal protein, eIF4a. It stimulates peripheral blood mononuclear cells from leishmania infected patients to proliferate and it produces a Th1 type of cytokine profile which down regulates IL-10 mRNA from both resting and activated cells. LeIF has also been observed to have exceptional adjuvant properties with a variety of antigens. It is a natural inducer of interleukin-12 (IL-12) in normal human PBMC. Its predicted molecular mass is 45kDa [13].

On *in vitro* testing, it was noted that this patient's T-cells did not recognize the four antigens TSA, LmSTI1, rLbhsp83 and LeIF. Because these antigens had previously shown strong T-cell responses *in vitro* when placed with cells from *Leishmania* infected mice and from other leishmaniasis patients, it was hypothesized that they might be important key proteins not recognized by the immune system of this patient. Because all of these antigens had negligible levels of endotoxin, as measured in the Limulus amebocytes assay, we prepared individual batches of Leishmanin skin test antigen with each of the four antigens according to our previously tested Leishmanin skin test antigen preparation procedures [14]. The concentration of the antigens was 50mg per ml for the antigens TSA, LmST11 and rLbhsp83, and 100mg per ml for LeIF.

The tolerability of these antigens was evaluated by recording allergic and DTH responses to each individual recombinant antigen by injection of 0.1 ml of each antigen intradermally at concentrations diluted 10x, 5x and 1x into 5 healthy volunteers after obtaining informed consent. No adverse reaction was recorded. DTH responses were recorded immediately, and at 24 and 48 hours after injection, but no reaction developed in the healthy subjects. The same safety evaluation of each individual antigen was performed using one patient with a single cutaneous lesion of leishmaniasis and the patient with refractory mucosal leishmaniasis following the same protocol. Neither patient exhibited a skin or systemic reaction to the highest dose tested. Therefore, the cocktail vaccine with the mixture of these four antigens was tested.

Tolerability was assessed in the same healthy volunteers using the same dose escalating protocol except that the injection route was subcutaneous instead of intradermal. 5mg of each individual antigen (TSA, LmSTI1, rLbhsp83) and 10mg of LEIF were the doses selected to prepare the vaccine to be used for the immunotherapy for this mucosal leishmaniasis patient. No reaction to the cocktail was observed except in one healthy subject who had erythema and induration at the injection site considered to be a secondary infection, but possibly due to an intense DTH response. Based on the good tolerability profile in disease-free individuals and the hypo responsiveness to the antigens in the mucosal leishmaniasis patient, this cocktail was selected to immunize our study patient.

The final formulation of the vaccine contained 5mg of the antigens TSA, LmSTI1, rLbhsp83 and 10mg of LEIF**.** All individual antigens were stored at -80ºC in sterile vials in the amount of 50mg, until use. The vials were thawed and used only once at each time of immunization. 50mg of GM-CSF (Leukine®) was added as adjuvant.

Adjuvant selection

The recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) has been documented to have a potent cytokine adjuvant effect based on its activity of inducing activation and migration of dendritic cells [15]. It has been shown to be effective in doses of 25mg to 50mg as an adjuvant [16, 17], and it has been shown to be well tolerated when used in patients with visceral leishmaniasis [18].

Immunization schedule

A few minutes prior to injecting the vaccine cocktail, individual batches of the antigen were thawed and the appropriate amount was mixed in a 2ml sterile vial under laminar flow hood sterile environmental conditions. The prepared vaccine cocktail was administered by subcutaneous injection in a volume of 0.5 ml in the anterior face of the forearm. Three doses of the vaccine were given a month apart. All applications of the vaccine were done in the hospital and the patient remained under observation for two hours and returned 24 and 48 hours later for the monitoring of adverse reactions.

Follow-up skin testing for specific antigen recognition

In addition to the clinical evaluation of response reported in the case report, skin DTH tests to the four antigens were done at 30 days after treatment. Each skin test showed induration as follows: TSA 20mm, LmSTI1 17mm, Lbhsp83 35mm, and LEIF 10mm. Thus, delayed-type hypersensitivity recognition to each of these antigens was documented.

Discussion

This case demonstrates that severe mucosal leishmaniasis refractory to antimonial therapy can be successfully treated with a cocktail of selected recombinant leishmania antigens and a cytokine (GM-CSF) adjuvant, yielding a well-tolerated vaccine.

The first line approach for the treatment of leishmaniasis is administration of pentavalent antimony compounds (4). Often, serious adverse reactions occur such as cardiac arrhythmias, severe arthritis, liver dysfunction, lethargy and, eventually, sudden death (5). Historically, thousands of leishmaniasis patients treated with antimonials are successfully cured, but always with the danger of well-documented side effects of heavy metal poisoning [19]. In addition, emergence of leishmanial resistant organisms to the pentavalent antimony is well documented and, in some endemic areas of the world, treatment failure has reached a level of 60% to 80% [20]. Unfortunately, second line alternative drugs are more toxic than antimonial compounds [3]. Amphotericin B and Pentamidine have shown reasonably good efficacy results in a series of cases reported, but both have been associated with severe, life threatening organ dysfunction and death [4, 5]. During the last decade, new formulations of Amphotericin B in a liposome or other lipid-complex drug delivery system have significantly decreased the side effects of Amphotericin based therapy [21]. However, the price of the liposome-Amphotericin B preparations is prohibitive for most of the millions of people with leishmaniasis in the tropics [22]. In addition, many reports of dramatic resistant or refractory cases of leishmaniasis, such as the one reported here, leave us with an unsolved challenge [23, 24].

Leishmaniasis is a well-known model of cell-mediated, protective immune responses to specific *Leishmania* antigens [25]. Each clinical manifestation of *Leishmania* infection has a different immunological picture. Patients with cutaneous leishmaniasis have strong delayed hypersensitivity and *in vitro* proliferative responses that occur during both active and cured disease [26]. Diffuse cutaneous leishmaniasis is characterized by uncontrolled cutaneous lesions in the absence of delayed hypersensitivity or T-cell proliferation responses to the parasite [27, 28]. Mucosal leishmaniasis is characterized by hyperactive intradermal skin tests and lymphocyte proliferative responses [29] that may explain the destructive attack on host tissue and the paucity of parasites in mucosal lesions [27]. Patients with acute visceral leishmaniasis lack *Leishmania*-specific delayed hypersensitivity (DTH) responses when specific antibody titers are high, and their lymphocytes fail to proliferate to the parasite antigens *in vitro* [29]. However, these patients become responsive after resolution of their symptoms [30]. In recent years, significant progress has been made in understanding the host control mechanisms responsible for this varied immunological picture in experimental models and in humans. Major advances contributing to our understanding of leishmania disease include delineation of Th1/Th2 responses [31, 32]; the definition of sub-clinical and asymptomatic infections [33]; the introduction of immunotherapy [6, 7, 34, 35];

and the identification and evaluation of several important leishmania genes and their related antigen genes [36, 37]. Immune-modulation as a therapeutic approach to the treatment of leishmaniasis is as old as the discovery of the *Leishmania* organism. Row, *et al*., recorded the use of immunotherapy in 1912, when no other therapy was available [38]. Use of soluble *Leishmania* antigen extracted from whole promastigote parasites, or a soup of several different promastigote parasite strains, as *Leishmania* vaccine antigen for immunization or immunotherapy, has been recorded many times since 1943 [39]. In a randomized trial, a combination vaccine consisting of live BCG together with killed *Leishmania* promastigote was compared with standard antimonial treatment in 94 patients with localized cutaneous leishmaniasis. Vaccination during a period of 32 weeks gave a cure rate (94%) similar to three 20-day courses of meglumine antimoniate [9]. In another open trial in Brazil, immunotherapy with a mixture of five stocks of *Leishmania (L. mexicana*; *L. amazonensis*; *L. (Viannia) guanensis; and two Leishmania complexe sp strains)* was used to prepare a heat-inactivated antigen with a 240mg/ml of protein content plus BCG to treat patients with multiple cutaneous and mucocutaneous leishmaniasis [7]. Seven of eight patients with multiples cutaneous lesions were clinically cured and six of eight with mucocutaneous leishmaniasis were successfully treated with this immunotherapeutic approach. Most of the patients required five to six courses of 10 consecutive days of injections of the vaccine preparation. Overall, the length of immunotherapy was six to eight months. The patient reported here received this same vaccine preparation during his six years of illness, but with no amelioration of his lesions. There is a question whether he had insufficient exposure to the particular antigens that might promote the healing effect, or whether the mixture of so many different antigens could have blocked the T-cell epitopes that might have triggered an effective Th-1 type of response. In leishmanial murine models, it has been well demonstrated that the type of antigen presented drives the Th 1 or 2 type of response [40].

Another consideration in the successful outcome of this patient is whether the selection of the cytokine adjuvant, GM-CSF, played a special role. This cytokine is known to yield a dominant Th1 type response, so one could consider that its use favored this immune response direction unrelated to the type of leishmanial antigen used. One must also consider the wound healing effects that might be promoted by the GM-CSF on the mucosal lesions themselves, although this would be very unlikely in view of the low dose (50mg) of the cytokine given at a site distant from the inflammatory lesion. It is of interest, however, that GM-CSF used in higher doses injected at the site of the lesion and in combination with pentavalent antimony, shortens the healing time [41].

Another important question is what immunological mechanism allowed this cocktail vaccine to rapidly promote the healing process of the mucosal lesions.

Th1 and Th2-cell subsets are differentially activated by macrophages and B cells in murine leishaniasis [42]. LeIF, one of the antigen components of the cocktail vaccine, has the ability to influence the Th1/Th2 cytokine response. In naive BALB/c mice immunized with LeIF, the T-cell clone derived preferentially secretes IFN-g. It also generates specific Th1 T-cell clones in the absence of adjuvant in SCID mice [13].

TSA is also a potent inducer of Th1 type cytokine responses. PBMC from our patients prior to the immunotherapy did not recognize this antigen, but there was a strong proliferative response after the second and third dose of the vaccine. Absence of proliferative cell responses in naive patients with active mucosal leishmaniasis was also demonstrated in two patients in the original report of the discovery of TSA [10].

However, LmSTI1, in spite of its Th1 preferential T-cell clone induction, has no inherent ability to drive Th cell differentiation. Immunization of BALB/c mice with LmSTI1 results in generating T-cell clones Th1, Th2, and Th0 type cytokine profiles. It is possible that the role of this antigen is to potentiate the already expanded clone [11].

Another question to be answered is what the role of Th2 cytokine responses is in the modulation of inflammation seen in the mucosal lesions.

IL-4 and IL-10 mRNA was found abundant in local T-cell tissue from the mucosal lesions of patients with active mucosal leishmaniasis [43]. The rh-hsp83 antigen stimulates a mixed Th1-Th2 pattern, and may act as a

key modulator in this unknown mechanism that promotes the control of this chronic inflammatory process in the MCL patients [12].

The complex balance of microbial replication and, perhaps, misdirected immunological response in each patient with recurrent, active mucosal leishmaniasis, may show that correction of the immune response can only be successful following, or concurrent with, proper antimicrobial action against the proliferating protozoa. If this were true, the repeated courses of therapy in this patient may have set the stage for successful solution of the immunological defect by immunotherapy.

One question will always recur, and that is how far a physician can go in seeking alternate therapy for patients suffering from apparently incurable diseases. There is no doubt that medical history is filled with well-intended, but, in retrospect, worthless medicines, provided by caring and innovative physicians as well as some memorable successes. The ethical issues related to any "experimental" approach require that the physician or physicians involved follow a standard set of guidelines. These guidelines include: 1) All aspects of risks and benefits resulting from an innovative approach must be carefully balanced so that the benefits outweigh the risks to the patient; 2) Physicians must ensure that a patient is able to truly understand the proposed treatment and is fully informed of what is being planned, the risks, and the benefits, and is truly informed and consenting; 3) Physicians must carefully examine any personal bias, other than the patient's health, that might influence the decision to proceed with treatment, and ensure that they have found none; 4) Any potential adverse reactions must be well studied before exposing the patient to risk, including if necessary, as was done in this case, self-administration of the vaccine to the physicians prior to treating the patient. The physicians caring for a patient must be confident that each of these criteria are met.

Although initially anxious because of the experimental nature of the treatment, in retrospect, our joyful patient provided sufficient comfort regarding any risks that might have occurred (but did not) during the resolution of a chronic, recurrent, and disfiguring disease such as mucosal leishmaniasis.

References

1. Marsden P.D. Mucosal Leishmaniasis ("espundia" Escomel, 1911. Trans R Soc Trop Med Hyg **1986**;80: 859-76.
2. Berman J.D. Human leishmaniasis: clinical diagnostic and thermo therapeutic developments in the last 10 years. Clin Infect Dis **1997**;24:684-703.
3. Sampaio S.A.P., Castro M., Dillon N.L., Martins J.E.C. Treatment of mucocutaneous (American) leishmaniasis with Amphotericin B: Report of 70 cases. Inter J Dermatol **1971**;10:179-81.
4. Herwaldt B.L. Leishmaniasis. Lancet **1999**;354:1191-9.
5. Pearson R.D., Jeronimo S.M.B., Sousa A.Q. Leishmaniasis. Tropical Infectious Diseases Principles, Pathogenesis, and Practice. Chapter **1999**;74(1):797-813.
6. Convit J., Castellanos P.I., Utrich M., et al. Immunotherapy of localized, intermediate, and diffuse forms of American Cutaneous Leishmaniasis. JID **1989**;160(1):104-15.
7. Mayrink W., Magalhães P.A., Michalick M.S., et al. Immunotherapy as a treatment of American Cutaneous Leishmaniasis: preliminary studies in Brazil. Parasitologia **1992**;34:159-65.
8. Genaro O., Toledo V.P., Costa C.A., et al. Vaccine for Prophylaxis and Immunotherapy, Brazil. Clinics in Dermatology **1996**;14(5):503-12.
9. Convit J., Castellanos P.L., Rondon A., et al. Immunotherapy versus chemotherapy in localized cutaneous leishmaniasis. Lancet **1987**;21:401-4.
10. Webb J.R., Campos-Netto A., Ovendale P.J., et al. Human and murine immune responses to a novel *Leishmania major* recombinant protein encoded by members of a multicopy gene family. Infect Immunity **1998**;66(7):3279-89.
11. Webb J.R., Kaufmann D., Campos-Netto A., Reed SG. Molecular cloning of a novel protein antigen of *Leishmania major* that elicits a potent immune response in experimental murine leishmaniasis. J Immunol **1996**; 157:5034-41.
12. Skeiky Y. , Benson D.R., Guderian J.A., et al. Immune responses of leishmaniasis patients to heat shock proteins of *Leishmania* species and humans. Infect Immunity **1995**;63(10):4105-14.
13. Skeiky Y., Guderian J.A., Benson D.R., et al. A recombinant *Leishmania* antigen that stimulates human peripheral blood mononuclear cells to express a Th1-type cytokine profile and to produce Interleukin 12. J Exp Med **1995**;181.
14. Reed S.G., Badaró R., Masur H., et al. Selection of a skin test antigen for American visceral leishmaniasis. Am J Trop Med Hyg **1986**;35:79-85.
15. Romani N., Gruner S., Brang D., et al. Proliferating dendritic cell progenitors in human blood. J Exp Med **1994**;180(1):83-93.

16. Lin R., Jones TC. Vaccine adjuvants: A new hope for efective immunizations. Braz J Infect Dis **1997**;1(3):106-22.
17. Tarr P.E., Lin R., Mueller E.A., et al. Evaluation of tolerability and antibody response after recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) and a single dose of recombinant hepatitis B vaccine. Vaccine **1996**;14(13): 1199-204.
18. Badaró R., Nascimento C., Carvalho J.S., et al. Recombinant human granulocyte-macrophage colony-stimulating factor reverses neutropenia and reduces secondary infections in visceral. JID **1994**;170:413-8.
19. The Leishmaniasis. Report of a WHO Expert Committee. World Health Organization Technical Report Series 701. World Health Organization [Eds.]. Geneva, Switzerland, pp. 1-140, **1984**.
20. Grogl M., Thomason T.N., Franke E.D. Drug resistance in leishmaniasis: its implication in systemic chemotherapy of cutaneous and mucocutaneous disease. Am J Trop Med Hyg **1992**;47(1):117-26.
21. Thakur C.P., Pandey A.K., Sinha G.P., et al. Comparison of three treatment regimens with liposomal Amphotericin B (AmBisome®) for visceral leishmaniasis in India: a randomized dose-finding study. Trans Roy Soc Trop Med Hyg **1996**;90:319-22.
22. Davidson R.N., di Martino L., Gradoni L., et al. Short-course treatment of visceral leishmaniasis with liposomal Amphotericin B (AmBisome). Clin Infect Dis **1996**;22:938-43.
23. Lira R., Sundar S., Makharia A., et al. Evidence that the high incidence of treatment failures in Indian kala-azar is due to the emergence of antimony-resistant strains of *Leishmania donovani*. JID **1999**;180:564-7.
24. Davidson R.N., Russo R. Relapse of visceral leishmaniasis in patients who were coinfected with human immunodeficiency virus and who received treatment with liposomal amphotericin B. Clin Infect Dis **1994**;19:560.
25. Reed S.G., Scott P. T-cell and cytokine responses in leishmaniasis. Current Opinion in Immunology **1993**;5:524-31.
26. Barral-Netto M., Badaró R., Barral A., Carvalho M. Immunology of cutaneous leishmaniasis. Rev Soc Bras Med Trop **1986**;19(3): 173-91.
27. Bittencourt A.L., Barral-Netto M. Leishmaniasis. In: *Spezielle pathologische Anatomie*. Begründer von Wilhelm Doerr und Erwin Uehlinger, Herausgegeben von W. Doerr und G. Suifert (Eds.). Vol. 8, 2nd Ed. "Tropical Pathology". **1995**; pp.597-651.
28. Carvalho E.M., Johnson Jr W.D., Barreto E., et al. Cell mediated immunity in American cutaneous and mucosal leishmaniasis. J Immunol **1985**;135(6):4144-8.
29. Carvalho E.M., Badaró R., Reed S.G., et al. Absence of gamma interferon and interleukin 2 production during active visceral leishmaniasis. J Clin Invest **1985**; 76(6): 2006-9.
30. Badaró R., Carvalho E.M., Orge M.G., et al. Imunidade humoral e celular em indivíduos curados de Leishmaniose visceral. Rev Soc Bras Med Trop **1985**;18 (2):77-83.
31. Uyemura K., Pirmez C., Sieling P.A., et al. CD_4^+ type and CD_8^+ type 2 T-cell subsets in human leishmaniasis have distinct T-cell receptor repertoires. J Immunol **1993**; 151:7095-104.
32. Mocci S., Coffman R.L. Induction of tH2 population from a polarized *Leishmania*-specific Th1 population by *in vitro* culture with IL-4. J Immunol **1995**; 154: 3779-3787.
33. Badaró R., Jones T.C., Carvalho E.M., et al. New perspectives on a subclinical form of visceral leishmaniasis. J Infect Dis **1986**;154(6):1003-11.
34. Badaró R., Johnson Jr W.D. The role of g-IFN in the treatment of visceral and diffuse cutaneous leishmaniasis. J Infect Dis **1993**; 167(Suppl. 1): S13-7.
35. Sundar S., Rosenkaimer F., Lesser M.L., Murray H.W. Immunochemotherapy for a systemic intracellular infection: accelerated response using interferon-gamma in visceral leishmaniasis. J Infect Dis **1995**;171(4): 992-6.
36. Myler P.J., Sisk E., McDonagh P.D., et al. Genomic organization and gene function in Leishmania. Biochem Soc Trans **2000**;28(5):527-31.
37. Skeiky Y.A., Kennedy M., Kauffman D., et al. LeIF: a recombinant Leishmania protein that induces an IL-12-mediated Th1 cytokine profile. J Immunol **1998**;161(11): 6171-9.
38. Row R. Curative value of *Leishmania* culture vaccine in Oriental sore. Brit Med J **1912**;9:540-1.
39. Dubovsky P. Vaccinotherapy of cutaneous leishmaniasis. Trop Dis Bull **1943**; 40: 297.
40. Schopf L.R., Bliss J.L., Lavigne L.M., et al. Interleukin-12 is capable of generating an antigen-specific Th1-type response in the presence of an ongoing infection-driven Th2-type response. Infect Immun **1999**;67(5):2166-71.
41. Almeida R., D'Oliveira A. Jr, Machado P., et al. Randomized, double-blind study of stibogluconate plus human granulocyte macrophage colony-stimulating factor versus stibogluconate alone in the treatment of cutaneous Leishmaniasis. J Infect Dis **1999**;180(5):1735-7.
42. Rossi-Bergmann B., Muller I., Godinho E.B. Th1 and Th2 T-cell subsets are differentially activated by macrophages and B cells in murine leishmaniasis. Infect Immun **1993**;61(5):2266-9.
43. Pirmez C., Yamamura M., Uyemura K., et al. Cytokine patterns in the pathogenesis of human leishmaniasis. J Clin Invest **1993**;91:1390-5.

lymphoma [15] or chronic hepatitis C [16], IL-2 is used in patients with AIDS [17], and numerous vaccine adjuvants have been developed [18]. The case recorded here of the use of immunomodulation in a patient with mucosal leishmaniasis is an important contribution, by applying recent knowledge of immunology to an ancient method of treatment.

Thomas C. Jones

References

1. Coley W.B. The treatment of malignant tumors by repeated inoculation of erysipelas: with a report of ten original cases. Am Jou Med Sci **1893**;105:487-511.
2. Roberts J.N. Jr. Impact of temperature elevation on immunologic defenses. Rev Inf Dis 1991;13:462-72.
3. Austin S.C., Stolley P.D., Lasky T. The history of malariotherapy for neurosyphilis. Modern parallels. JAMA 1992;268:516-9.
4. Garrison F.H. The seventeenth century: The age of individual scientific endeavor, in An Introduction to the History of Medicine. W.B. Saunders Co., p. 241-313, 1924.
5. Garrison F.H. The period of the renaissance, the revival of learning, and the reformation (1453-1600), in An Introduction to the History of Medicine. WB Saunders, p.185-240, 1924.
6. Lawrence H.S. Transfer factor in cellular immunity. Harvey Lect 1974;68:239-50.
7. Lugosi L. Theoretical and methodological aspects of BCG vaccine from the discovery of Calmette and Guérin to molecular biology. A review. Tuber Lung Dis 1992;73:252-61.
8. Zinkernagel R.M. Immunology taught by viruses. Science 1996;271:173-8.
9. Genaro O., deToledo V.P., da Costa C.A., et. al. Vaccine for prophylaxis and immunotherapy, Brazil. Clin Dermatol 1996;14:503-12.
10. Webb J.R., Kaufmann D., Campos-Neto A., Reed S. Molecular cloning of a novel protein antigen of Leishmania major that elicits a potent immune response in experimental murine leishmaniasis. J Immunol 1996;157:5034-41.
11. Lin R., Tarr P.E., Jones T.C. Present status of the use of cytokines as adjuvants with vaccines to protect against infection. Clin Inf Dis 1995;21:1439-49.
12. Lin R., Tarr P.E., Jones T.C. Considerations regarding the use of cytokines in active specific immunotherapy of cancer. Vaccine Research 1996;5:41-52.
13. Fauci A.S., Rosenberg S.A., Sherwin S.A., et al. Immunomodulators in Clinical Medicine. Ann Int Med 1987;106:421-33.
14. International Chronic Granulomatous Disease Cooperative Study Group. A controlled trial of interferon gamma to prevent infection in chronic granulomatous disease. N Eng Jou Med **1991**;324:509-16.
15. Engelhart M. Possible role for cytokines in the treatment of lymphoma. In Hemopoietic Growth Factors and Mononuclear Phagocytes, Ed. R. Van Furth, Karger, Basel, p. 177-86, **1993**.
16. Davis G.L., Balart L.A., Schiff E.R., et. al. Treatment of hepatitis C with recombinant interferon alpha. A multi-center randomized, controlled trial. N Eng Jou Med **1989**;321:1501-6.
17. Davey R.T. Jr, Chaitt D.G., Piscitelli S.C., et. al. Subcutaneous administration of interleukin-2 in human immunodeficiency virus type 1-infected persons. Jou Inf Dis **1997**;175:781-9.
18. Lin R., Jones T.C. Vaccine adjuvants: A new hope for effective immunizations. Braz Jou Inf Dis **1997**;1:106-16.