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Leishmania Amastigotes Induced Remission of Cutaneous Leishmaniasis, Psoriasis, Psoriatic Arthritis and Related Diseases

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Authors' contributions

This work was carried out in collaboration between all authors. Author JAO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author JG managed corrections in the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Amastigotes from *L(L) amazonensis (La); L(L) venezuelensis (Lv); L(V) brasiliensis (Lb)* and *L(L)chagasi(Lch)* were cultured axenically in a liquid culture medium (O'Daly's medium). Patients from a cutaneous leishmaniasis (CL) endemic and hyperendemic regions in Venezuela, receiving different treatments were followed up for 6 years. Remission of lesions (weeks) were: Spontaneous remission (SR): 7; Glucantime® (Glu) chemotherapy: 9; Immunotherapy with *La, Lv, Lb, Lch* amastigotes Tosyl-Lysil-Chloromethyl-Ketone (TLCK) treated and Nonidet P-40 (NP-40) extracted (AS100-1VT): 7. While vaccinating subjects for CL protection, we observed 100% clinical remission of a psoriatic lesion in one subject. In an open trial, 2,770 subjects showed baseline psoriasis area and severity index (PASI) compared with post-treatment values as follows: PASI 100, 23%; PASI 75, 45%; PASI 50, 13%; PASI 10, 9% and <PASI 10, 3% of patients, without serious adverse

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events. Similar results were obtained with AS100-2 *La, Lv, Lb* and *Lch* monovalent vaccines. After treatment with AS100-1VT, subjects with psoriatic arthritis (PsA) decreased in arthritis score, tender joints counts and nail changes; the highest decreased in the PASI 100 group. The vaccines induced cellular immunity with absence of humoral antibody responses. Lymphocyte subsets (LS) in peripheral blood mononuclear cells (PBMC) decrease as PASI increased migrating from the blood to the skin/joints. After vaccine treatment the LS migration is stopped explaining remission of lesions. Purified *Leishmania* antigenic fractions (AS200) induced linear delayed type hypersensitivity reactions (DTH) in guinea pigs. A DBA-1 mouse collagen induced arthritis (CIA) model with AS200 treatment had the least amount of forepaw inflammation and the lowest arthritis scores, lower than dexamethasone. The vaccines AS100-1VT, AS100-2 and AS200 were not immunosuppressors, but immunomodulators decreasing inflammatory cytokines. The aim of this review is to explain the serendipity discovery of leishmania parasites inducing remission of psoriasis and related diseases, unprecedented discovery in the scientific literature.

Keywords: Cutaneous leishmaniasis; amastigotes vaccine; psoriasis; psoriatic arthritis; rheumatoid arthritis.

1. INTRODUCTION

1.1 Epidemiology of Leishmaniasis

Leishmaniasis is a global zoonosis from the tropics and subtropics, with humans serving as accidental hosts. One-tenth of the world's population (700 million people) is at risk of infection. Globally, there are 12 million cases and the incidence of visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) infections are approximately 0.5 and 1-1.5 million new cases each year, respectively [1,2] Recent epidemiological data is found in World Health Organization Leishmaniasis Control home page: http://www.who.int/ctd/html/leis.html.

1.2 Host Response to Cutaneous and Visceral Leishmaniasis

The parasite strains in blood and skin were the same, confirmed by isoenzyme analysis [3]. The insoluble antigenic fraction from parasites primarily stimulated CD4+ T cells, while the soluble fraction showed a mixed profile, with CD4+ T cells being the main cell type responsible for Th2 cytokines and CD8+ T cells for Th1 cytokines [4,5]. Residual parasites remain in the host forever and can be reactivated by AIDS [6,7].The development of effective and affordable vaccines against leishmaniasis has not been achieved. Candidate antigens, including heat-killed promastigotes, live attenuated parasites, crude parasites, pure or recombinant *Leishmania* proteins or DNA encoding them, as well as immunomodulators from sand fly saliva have been used, however, very few candidate vaccines have progressed beyond the experimental stage [8,9]. Increased synthesis of Heat Shock Protein (HSP) occurs in prokaryotic and eukaryotic cells when they are exposed to stress, to protect themselves from lethality, and represent target antigens of the immune response [10].

After being bitten by the vector, vertebrates defend against CL with a granuloma composed of granulocytes, lymphocytes, epithelioid cells, monocytes, macrophages, and fibroblasts at the site of infection. In addition to killing parasites, activated macrophages produce different cytokines such as Tumor Necrosis Factor α(TNFα), interleukin(IL)-6, IL-18, IL-12, and IFNγ inducing a protective Th1-type immune response [11,12]. Analyses of immune responses in natural and experimental (healthy human volunteers) infections show that the clear Th1/Th2 T cell responses to *L(L)major* seen in murine studies do not occur. Instead, a typical mixed Th1/Th2 response is observed with PBMC from patients secreting varying amounts of IFNγ, IL-10, and IL-4 depending on the clinical stage of the disease. CD4+ and CD8+ T cells contribute to IFNγ and TNFα production in infected patients [13].

TNFα plays a central role in the defense against intracellular infections with Leishmania, a disease that ends fatally in TNFα –/– mice [14]. Resolution of an established infection is mediated by IFNγ produced by CD4+ T cells in C57BL/6 resistant mouse strains. The IFNγ *response* allows macrophages to develop leishmanicidal activities as expression of inducible nitric oxide synthase (iNOS) and NO. In contrast, BALB/c susceptible strain develops IL-4 and IL-10 mediated CD4+ T cell response. Rheumatoid arthritis (RA) patients treated with TNFα antagonists have reoccurrence of

leishmaniasis. After blocking TNFα, *L(L) donovani* infected mice were unable to resolve the infection [15].

VL is characterized by abundant parasites in the spleen, liver, and bone marrow. However, the parasite only establishes chronic infection in the spleen and bone marrow because infection in the liver is self-resolving within 6–8 weeks due to a Th1 dominated granulomatous response, characterized by high IFN-γ production. Neutrophils are rapidly recruited to the site of *Leishmania* inoculation, where they phagocytose the parasites, some of which are able to survive within these first host cells. Neutrophils can thus provide a transient safe shelter for the parasites, prior to their entry into macrophages where they will replicate [16].

1.3 Vaccination with Polyvalent, Monovalent and Isolated Proteins Vaccines from *Leishmania* Amastigotes Inducing Remission of CL, Psoriasis, Psoriatic Arthritis and Experimental Rheumatoid Arthritis

After vaccination with 3 doses of AS100-1VT vaccine at 500 µg/500 µl , one month apart in the endemic area of "Valle Arriba" and in the hyperendemic area of "La Planta" both in Guatire, Miranda State, Venezuela, protective efficacy were 85.9% and 71% respectively. The vaccine also induced regression of CL lesions when used as immunotherapeutic agent (Fig. 1A-1C). In the endemic area we found one person cured from plaque psoriasis one month after the third dose of vaccine, a serendipity finding. The study group sample (n=87) from "La Planta" a hyperendemic region for CL had prevalence per 100,000 subjects of 24.8 o/oo, exhibiting similar percentages of patients with scars (49.25%) and ulcers (49.25%) after primary infection while, 1.5% had skin nodules in uncovered areas from the body [17].

Treatments were distributed in 87 patients with primary lesions as follows: 35% had SR of lesions, 42% received Glu, and 21% a vaccine of amastigotes with the following Leishmania strains: *La, Lv, Lb* and *Lch*. Amastigotes were cultured axenically in O'Daly's liquid culture medium and incubated with TLCK and extracted with NP-40, a first generation polyvalent preparation named as AS100-1VT vaccine (Figs. 1A-1C). Second generation monovalent antigens (AS100-2 *La, Lv, Lb* and *Lch*) vaccines were prepared in the same manner with TLCK and NP40 as published [17]. Diagnosis was established by IDR > 5 mm with polyvalent leishmanine and enzyme linked immunoabsorbent assay (ELISA) with amastigotes antigens. Patients with IDR > 5 mm were selected, a biopsy taken with dermal punch of 6 mm in diameter, from the edge of the ulcer, and aliquots processed for microscopic analysis of amastigotes in macrophages after May-Grunwald-Giemsa staining, injection of 0.1 ml in mice footpads and parasite culture in O'Daly's medium a novel culture medium structured with 65 substances with concentrations selected after incubation of parasite extracts at 37 C for 48 hours and supplemented with 5% fetal bovine serum. An effective clinical remission was considered the total involution of ulcers or nodules, and the appearance of a retractile scar without any inflammatory signs and no relapse for a follow up period of 6 months after remission [17].

2. PATIENTS WITH REMISSION OF LESIONS WITH AMASTIGOTES AS100-1 VT TREATMENT

Seven weeks was chosen for remission time, selected from patients with natural SR of CL without any treatment. Patients treated with Glu had 9 weeks and with AS100-1VT, 7 weeks for remission. Two patients without remission post-Glu received 7±1 of AS100-1VT doses and remitted in 7 weeks. Patients that received AS100-1VT had 6 doses for total remission in 7 weeks, without relapses in 6 years of follow up [17].

3. FINDINGS IN PATIENTS WITH CL AFTER TREATMENT WITH AMASTIGOTES AS100-1 VT VACCINE

The IDR with polyvalent AS100-1 VT had a diagnostic value >1.0 for CL surveillance useful to know the prevalence and incidence in endemic and hyperendemic regions. The association of protective immunity with IDR response and cellular immunity, explains the higher incidence of CL in IDR- (59%) as compared with IDR+ (27%) volunteers in the study group [17]. The basis of AS100-1VT vaccine was inhibition of serine proteases with TLCK, and extraction with NP40 to discard surface proteins that were not used, treatments which induced immune prophylaxis and immunotherapy in CL patients.

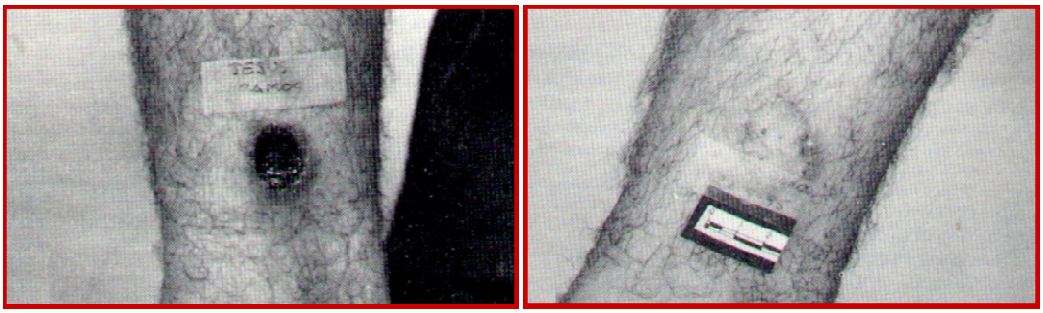

Fig. 1A. Male 14 years old. Ulcer in right leg: 8.75 cm^2 with evolution time: 1.5 months. AS100-1VT vaccine 22 doses; periodicity: weekly. Remission time: 5 months. Relapse none in 6 years of follow up. Adapted from reference 21

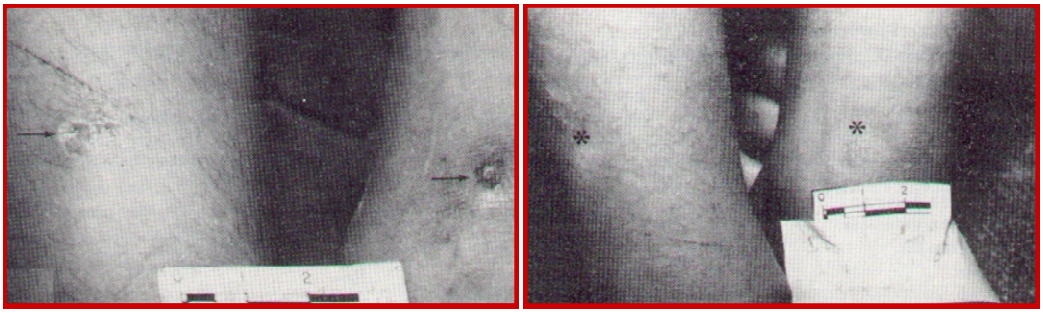

Fig. 1B. Male 9 years old. Ulcer in knees: 1.0-1.5 cm^2 with evolution time: 1.5 months. AS100-1VT vaccine 4 doses; periodicity: weekly. Remission time: 1 months. Relapses: none in 6 years of follow up. Adapted from reference 21

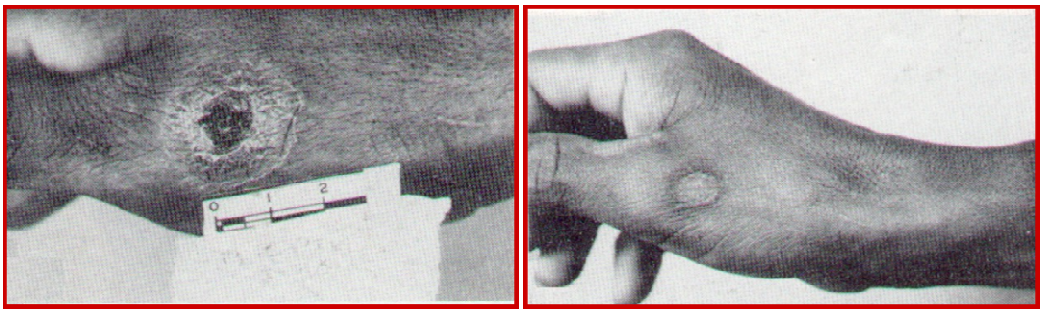

Fig. 1C. Male 30 years old. Ulcer in right hand: 3.14 cm^2 with evolution time: 2 months. AS100-1VT vaccine 14 doses; periodicity: weekly. Remission time: 3.5 months. Relapses: none in 6 years of follow up. Adapted from reference 21

Specific antibodies decreased post-clinical remission of lesions, confirmed by ELISA, band frequency and integral optical density (IOD) band area in immunoblottings, pointing out the fundamental role of cellular immunity in controlling infection. Size of skin ulcers in relapses was lower than in primary lesions thus, a vaccine was possible to control CL. AS100-1VT vaccine had remission time similar to natural SR; evidence it was useful for CL treatment. Furthermore AS100-1VT vaccinated subjects had lower CL cases in IDR- and IDR+ volunteers than IDR- non-vaccinated subjects. ELISA in active lesions, pre-SR and pre-VT was similar in primary infection evidence that B cell clones were activated and reacting to all monovalent amastigote antigens. Antibodies decreased significantly post-SR, post-VT and post-Glu treatment, since AS100-1VT was stimulating mainly cellular immunity. Interestingly antibodies did not decrease in one parallel group of patients without clinical remission post-Glu treatment [17]. Phase I experiments were done in hamsters immunized with TLCK treated NP40 extracted Lb amastigotes from culture, and subsequently infected with Lb amastigotes isolated from

hamsters footpad. The animals showed an increase in T and B cell responsiveness to mitogens by lymph node lymphocytes, no changes in response for dextran sulfate and pokeweed mitogen in splenocytes, absence of parasites in lymph nodes after 6 weeks post-infection and a nodule 4 times smaller than that of infected control animals; which was undetectable 70 days post-infection. Hamsters pre-immunized with TLCK treated NP40 extracted *Ld* amastigotes from culture and subsequently infected with spleen *Ld* amastigotes survived for more than one year, whereas infected, unimmunized animals died five months after infection. Animals pre-immunized with culture parasites (*Lb* or *Ld*) treated with phenyl-methyl-sulphonyl-fluoride (PMSF) and infected with *Lb* or *Ld* amastigotes from infected hamsters did not show any protective effect [18].

The effect of *Leishmania* vaccine can be explained by *Leishmania* promastigotes lysates inducing *in vitro* proliferation and IFNγ production in PBMC from individuals that never had contact with *Leishmania* parasites. The proliferating T cell population was CD2+ in a frequency <1:10,000 a response that could be abolished after depletion of CD45RO+ memory cells from the PBMC [19]. Thus, normal CD2+ T cells and normal immunoglobulin from healthy volunteers that never experienced *Leishmania* infection reacted with *Leishmania* antigens in the immunoblottings assay. Interestingly psoriatic patients, DTH negatives to amastigote antigens, also recognized in a blastogenic assay *Leishmania* antigens and were distributed in two groups, low and high responders to amastigote antigens before treatment [20]. Furthermore, a high DTH response with *Lb* and *Lch* protein DEAE chromatography fractions *in vivo* after treatment with AS100-1VT polyvalent vaccine was found in human volunteers, confirming the absence of immunosuppression. The lymphocyte stimulation indexes obtained by protein fractions were 50-70% lower than the values induced by intact living *Leishmania* amastigotes. This suggests *in vitro* stimulation appears to be selectively focused toward a particular subset of lymphocytes which may be regulatory CD8+ T cells [20].

How can we reconcile the finding that treatment with an amastigotes antigen vaccine targeted at leishmaniasis, results in clinical remission of psoriasis in subjects never exposed to *Leishmania* parasites? Despite some of the obvious differences between the diseases, there are some striking similarities: immunity against both diseases starts with the same cell types, and also both diseases have similar Th1 cytokine patterns at least initially, both diseases respond to the *Leishmania* polyvalent vaccine AS100-1VT. It should be noted that both trials showed evidence of cellular immunity not humoral immunity. In psoriasis, initial interaction of antigens between APC and T cells occurs in the lymph nodes. Subsequent psoriatic antigen presentation occurs in psoriatic plaques that structurally resemble a lymph node, but are found in the skin. At this stage of psoriatic disease, the cellular conformation is similar to the granuloma found in the skin after *Leishmania* infection [21].

4. DISCUSSION

4.1 Psoriasis Clinical Features

Psoriasis is a systemic chronic, relapsing inflammatory skin disorder, with worldwide distribution, affects 1–3% of humanity, prevalence varies according to race, geographic location, and environmental factors [22]. The overall rate of comorbidities in subjects with psoriasis aged less than 20 years old was twice as high as in subjects without psoriasis. Juvenile psoriasis was associated with increased rates of hyperlipidaemia, obesity, hypertension, diabetes mellitus, Crohn disease and RA. In psoriatic patients, there is a 50% increase in cardiovascular death compared with the general population The best-known noncutaneous condition associated with psoriasis is joint disease, mostly expressed as psoriatic arthritis (PsA), [23]. Palm-plantar psoriasis is associated with significant quality of life issues. In 150 patients with palm-plantar psoriasis, 78 (52%) patients displayed predominantly hyperkeratotic palm-plantar lesions, 24 (16%) pustular, 18 (12%) combination of both, and 30 (20%) had an indeterminate phenotype. In 27 (18%) patients, lesions were confined to the palms and soles. In all, 27 (18%) had mild, 72 (48%) moderate, and 51 (34%) severe disease involvement [23].

Two forms of psoriasis can be recognized: Type I psoriasis, characterized by hereditary, dominant autosomic (60% penetration), onset: 16 years old females, 22 years old males; HLA-Cw6 positive (73.8% vs. 20.4% in normal subjects). Type II psoriasis, characterized by been sporadic, major incidence 57-60 years, poor correlation with HLA-CW6 (27.3% vs. 10.1% in controls). Psoriasis plaques with silvery scales present the

Auspitz sign a pinpoint capillary bleeding when the scales are gently scraped away with a spatula or fingernail [24,25]. Psoriasis may also attack nails], tendons, ligaments, fascia, and spinal or peripheral joints as the clinical form, inflammatory PsA similar to RA, but no rheumatoid factor present in the blood. PsA can be severely disabling, occurring in up to 10–30% of patients with psoriasis, and is associated with HLA-B27 MHC Class I marker [23].

4.2 Psoriasis Pathogenesis

The new concept of psoriatic disease permits a better understanding of psoriasis pathogenesis and comorbidities [26,27]. The disease is genetically determined by multiple genes that interact with each other and with environmental factors [28,29,30]. In early lesions, macrophages are present in the epidermis followed by monocytes, lymphocytes, and granulocytes with formation of spongiform micro abscesses (Munro abscesses), more pronounced with disease activity, a hallmark of psoriasis. Physical trauma to the skin, results in a psoriatic lesion (Koebner phenomenon), which increases when the disease is active [23]. The inflammatory process is immune mediated by unknown antigens through binding and specific activation and costimulation of T cells by antigen-presenting cells (APC), dendritic cells (DC), and macrophages in epidermis and dermis. A multimolecular complex is formed between APC and T cells: the immunological synapse, structured by major histocompatibility complex (MHC) receptors and T-cell receptors (TCR), with the following costimulatory molecules: lymphocyte functional antigen (LFA-1 and LFA-3), intercellular adhesion molecule (ICAM-1), and cluster of differentiation (CD)2, CD28, CD80, [26]. Epidermal keratinocytes are highly active immunological cells, controlling the acute and the chronic phase of skin inflammation by cytokine/chemokine production and surface molecule expression, which lead to inflammatory infiltrate in the whole skin including the upper layers of the epidermis, perpetuating the skin disorder [31].

Autoantibodies have been reported in psoriasis patients. Anti-nuclear antibody and antibody to double-stranded deoxyribonucleic acid, rheumatoid factor and anti-thyroid microsomal antibody (anti-TMA) were studied. About 28.8% of psoriasis cases were positive for at least one autoantibody. Age of onset and types of psoriasis had significant association with gender. Anti-double-stranded DNA and anti-TMA had significant association with types of psoriasis. Gender wise distribution of psoriasis in age group had significant association with anti-TMA. Autoantibodies are found in psoriasis patients and latent autoimmune diseases develop in psoriasis patients without any clinical symptoms [32]. Interestingly antibodies were not detected after vaccination with AS100-1 VT *leishmania* vaccines.

Angiogenesis is essential for embryo development, for wound healing and progression of a number of diseases such as cancer, inflammatory conditions, eye diseases, psoriasis, and RA in the adult. Current paradigms explain blood vessel growth entirely by sprouting angiogenesis or by vessel splitting. Blood vessel growth in the adult can be induced and directed by mechanical forces that naturally develop during healing or remodeling of tissues [33]. It is regulated by pro- and anti-angiogenic molecules, and was only implicated in few diseases, such as, cancer, arthritis and psoriasis, now its research offers a potential to cure a variety of diseases such as Alzheimer's and AIDS [34]. Angiogenic factors, such as vascular endothelial growth factor (VEGF), may dominate the activity of anti-angiogenic factors and accelerate angiogenesis in psoriatic skin. Small peptides with homologies to pigment epithelium derived factor (PEDF) show anti-angiogenic potential for the topical treatment of psoriasis. The specific low molecular weight peptides (<50 kDa) penetrated the skin and showed significant anti-angiogenic activity in vitro [35,36]. Similar peptides have been found in amastigotes AS100-1 VT vaccines. The first clinical sign of regression of lesions was decreased redness in psoriatic plaques. Psoriasis is associated with chronic inflammation and it often coexists with inflammatory arthritis in which IL-33 has been implicated [37]. IL-33 is one of the newest members of the IL-1 family of inflammatory cytokines [38] and can mediate IgE-induced anaphylaxis in mice [39]. IL-33 also induces release of IL-6 from mouse bone marrow-derived cultured mast cells [40] and IL-8 [41]. IL-33 augments SP-stimulated VEGF release from human mast cells and IL-33 gene expression is increased in lesional skin from patients with psoriasis [42]. Macrophage migration inhibitory factor (MIF) is implicated in a range of pathological conditions, including asthma, RA, atherosclerosis, inflammatory bowel disease and cancer. MIF is believed to be a detrimental factor in diseases such as systemic sclerosis, atopic

dermatitis, psoriasis, eczema and UV radiation damage [43].

Factors such as climate, physical trauma, drug, stress and infections such as *Streptococcus* and human immunodeficiency virus are known to trigger psoriasis. By immune-staining for IL-17A and IL-22, it has been shown numerous cells present in psoriasis lesions that produce these cytokines. Th17 cytokines (IL-17A, IL-22), and TNFα markedly increased the expression of CC chemokine ligand (CCL)-20, and also CC chemokine receptor (CCR)-6 ligand, in human keratinocytes monolayer and raft cultures in a dose and time dependent manner [44].

Production and uptake of inducible HSP70 by keratinocytes may critically influence the chronic course of inflammatory skin diseases. Human keratinocytes release high levels of inducible HSP70 that enhances peptide uptake. The stress-inducible chaperone HSP70 is considered a 'danger signal' if released into the extracellular environment. It has been proposed to play a role in the pathogenesis of skin diseases such as psoriasis and lupus erythematosus (LE). Living keratinocytes are an important source of HSP70 in the skin and release more HSP70 than fibroblasts, macrophages or lymphocytes. Keratinocytes also bind and internalize HSP70–peptide complexes; a process enhanced by TNFα and IL-27. No difference with regard to HSP70 release or uptake was observable between keratinocytes from healthy donors or patients with cutaneous LE. Keratinocytes pulsed with HSP70–peptide complexes significantly increased IFNγ production by autologous T cells which influence the chronic course of inflammatory skin diseases [45]. HSP60 and HSP70 peptides have been found in amastigotes AS100-1 VT vaccines.

4.3 Psoriasis and the Nervous System

Neuropeptides especially substance P (SP) [46] are involved in the pathogenesis of psoriasis. In particular, SP reactive fibers are localized close to mast cells [47]. SP can stimulate mast cells [48] and contributes to inflammation. SP-positive nerve fibers are denser in psoriatic lesions and have an increased number of mast cell contacts compared to normal skin [49]. The neuronal contribution to psoriasis at the remission and exacerbation phases were analyzed by the expression of the neuronal markers protein gene product 9.5 (PGP 9.5), growth associated protein-43 (GAP-43) and substance P, in addition to its receptor (R), neurokinin-1R (NK-1R) in psoriatic skin. The number of epidermal PGP 9.5 immuno-reactive nerve fibers in the involved skin during exacerbation was decreased compared to involved skin at remission and non-involved skin at the exacerbation phase. GAP-43-positive nerve fibers were decreased in the involved skin in contrast to non-involved skin, during exacerbation. Substance P expression was seen on both immuno-reactive nerve fibers and cells with a down-regulation in the number of positive nerve fibers in the involved skin compared to non-involved skin, at the exacerbation phase. The number of substance P-positive cells was slightly lower in the involved skin at exacerbation than at remission. The number of NK-1R immuno-reactive cells was increased in the involved skin in contrast to non-involved skin, at the exacerbation phase. These findings suggest a crosstalk between the nervous system and inflammation during psoriasis exacerbation in the form of an altered expression of nerve fibers, substance P and its NK-1R [50].

NGF is a key mediator of inflammation and pain. NGF influences an inflammatory reaction by regulating neuropeptides, angiogenesis, cell trafficking molecules, and T cell activation [51]. Psoriasis is characterized by keratinocyte hyper-proliferation and reduced apoptosis, leading to an increased epidermal turnover. Interestingly, NGF is both a mitogen and a survival factor for keratinocytes, is over-expressed in psoriatic lesions as well as in psoriatic keratinocytes, [52] and its high-affinity receptor TrkA, that is located only in basal keratinocytes in healthy skin, is expressed throughout all epidermal layers in psoriasis [53]. On the other hand, P75NTR that plays a pro-apoptotic role in keratinocytes, is absent in psoriatic keratinocytes. The rate of apoptosis in psoriatic transit amplifying (TA) cells is significantly lower as compared to TA cells from normal epidermis. On the contrary, in psoriasis, NGF and Trk up regulation associated with reduced P75NTR expression result in increased keratinocytes proliferation and reduced apoptosis, thus favoring epidermal thickness, a typical feature of this dermatosis [54].

Plaque symmetry, stress-induced onset or exacerbations, pruritus, and possibility of generalization, suggest a role of the nervous system and neurogenic inflammation in psoriasis pathogenesis. A key to understanding the role of melanocyte in psoriasis is their ability to act as regulatory cell in maintaining epidermal homeostasis. The disease probably begins with

so far unknown signal directed through neuronal network to the melanocyte, placed in the center of epidermal unit. That signal governs keratinocytes cellular activities and lead to reactive abnormal epidermal differentiation and hyper-proliferation. Increased proliferation of basal keratinocytes and high metabolic demands creates angiogenesis in papillary dermis and elongation of dermal papillae. Stimulated melanocytes and basal keratinocytes become an important source of pro-inflammatory cytokines that attract lymphocytes into the dermis [55]. Pictures with melanocytes in cured patients have been presented in this paper (Figs. 2-4).

Fig. 2. Female, 29 years old. Two years with plaques. Previous treatment: Topic steroids, methotrexate, Cuban treatment, coal tar, Vitamin D derivatives, Vitamin A + Vitamin D topical derivatives . Triggers: Ethanol, stress, diet. Initial PASI: 14.1(06-11-1999). Final PASI: 1.7 (12-03 1999).Doses/periodicity: 15/ every 10 days. Time: 6 months. Relapses: none

Psoriasis, has been named as the itch that scales; its importance is shown by the observation that sensory denervation leads to plaque resolution. Characteristic areas of psoriatic itch are the buttocks, extensor surfaces of the knees and elbows, and the ears and scalp. In psoriasis, 41–80% of patients have daily itch. Neuropeptide Y is inhibitory with respect to itch and decreased levels are seen in patients with psoriasis, which may explain the increased pruritus in psoriasis. Itch description varies in patients with psoriasis, ranging from stinging to burning to itch that affects sleep [56,57]. Amastigotes treated TLCK+NP40 antigens might also increase neuropeptide Y since the first symptom after AS100-1 VT injection is itch disappearance which permit the patient to go to sleep. Psoriasis is a systemic disease of the skin, nails, and joints, with a complex genetic basis. Early genome-wide linkage studies of psoriasis focused on segregation of microsatellite markers in families; the only locus consistently identified resided in the MHC, HLA-Cw6 itself to be the major susceptibility allele. A collaborative genome wide association study of psoriasis involving thousands of cases and controls revealed association between psoriasis and seven genetic loci: HLA-C, IL12B, IL23R, IL23A, IL4, IL13, TNFAIP3, and TNIP1 [58].

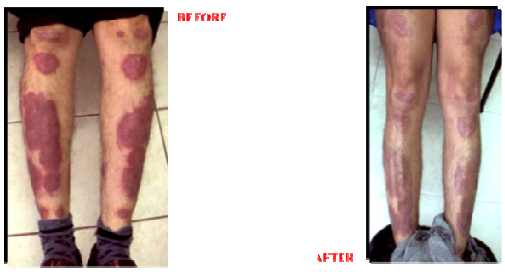

Fig. 3. Male, 24 years old. Four years with plaques. Previous treatment: Topic steroids. Initial PASI: 28.8(08-12-1997). Final PASI: 2.4 (12-01-1999). Doses/periodicity: 36/ every 30 or 15 days. Time remission: 28 months. Relapses: none. Relatives: cousin

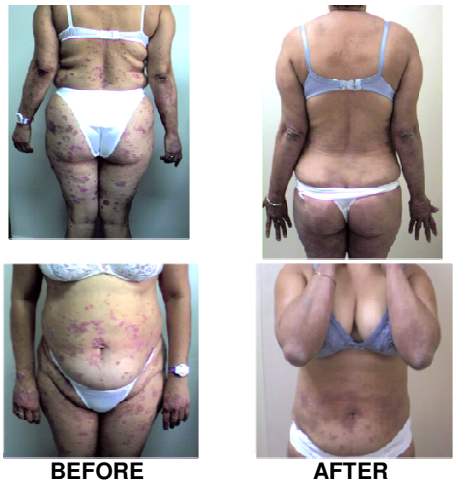

Fig. 4. Female, 42 years old. 17 years with plaques + Gutatta. Previous treatment: Topic, oral, intramuscular, intradermic steroids, ketazol, coal tar, acupuncture. Triggers: Ethanol, stress, diet, tobacco. Initial PASI: 25.5(10-07-1999). Final PASI: 0.2 (06-13-2000). Doses/periodicity: 16/ every 15 days. Time remission: 8 months. Relapses: none. Relatives: cousin

4.4 *Leishmania* Amastigotes Inducing Remission of Psoriasis a Serendipity Finding

While treating subjects in Venezuela with a vaccine containing *Leishmania* amastigotes antigens for prevention of CL, we observed 100% clinical remission of a psoriatic lesion in

one subject, a natural double blind serendipity finding. A first generation polyvalent vaccine (AS100-1VT) was manufactured with amastigotes from four axenically cultured *Leishmania* species. Parasites from *La, Lv, Lb* and *Lch*, each species at a ratio 1:1:1:1 were injected at 500 µg/ dose in 0.5 µl intramuscularly in the deltoid for three times one month apart [17]. PCR reactions confirm the absence of parasite DNA, in the polyvalent AS100-1VT vaccine. Lipophosphoglycan (LPG) is a glycoconjugate present on metacyclic promastigotes, which functions as a virulence factor in all *Leishmania* species. The final product of amastigotes extracts after TLCK treatment and NP40 surface antigen extraction, had 10 ng/ml or less of LPG a negligible amount. The acceptable endotoxin limit for the vaccine was 700 EU/ml. The vaccine had <50 EU/ml but >25 EU/ml. well below the acceptable limit. BSA was between 12.5 and 25.0 ng/ml, evidence that no fetal bovine serum (FBS) proteins from the culture medium were present. SDS acrylamide gels of AS100-1VT vaccine under reducing conditions exhibited 23–30 bands from 112.0 to 10.0 kDa molecular weight in the four *Leishmania* species; 21 bands (70%) with similar molecular weights (value variations 1% or less) in all lots. The percent homology between lots of the same specie was La 96.6%; Lb 86.7%; Lch 95.8%; Lv 91.3% [56]. A double-blind, placebo-controlled, parallel group study, of multiple doses of AS100-1VT was performed in 2,770 volunteers and included plaque (79%), gutatta (10%), plaque and gutatta (10%), palm/plantar (0.3%), erythrodermia (1.8%), inverse (0.8%), plaque and arthritis (3.4%) and nail psoriasis (0.3%).

Efficacy of *Leishmania* amastigotes vaccines was assessed by performing skin examinations and recording psoriasis area and severity parameters at each visit. The primary efficacy parameters were the percentage reduction in PASI score at each visit and the comparative proportions of subjects with 100, 75 and 50% PASI improvement in each treatment group. The lesions and the extent of body surface area involved were measured separately for the head (Ah), trunk (At), upper extremities (Au) and lower extremities (Al). The PASI combines lesion measurements of the skin erythema, (E, redness), skin induration (I, thickness) and skin desquamation (D, scaliness) of the lesions. Each sign in the lesion was quantified as follows: 0 none, 1 slight, 2mild, 3 moderate and 4 severe. The extent of body surface area affected was evaluated as follows: 1 < 10%, 2 = 10–30%, 3 = 30–50%, 4 = 50–70%, 5 = 70–90%, 6 > 90%. For evaluation purposes, the weighted contribution of each section of the body to the total body surface area is as follows, the head 10%, the thorax 30%, the upper extremities 20% and the lower extremities 40%. To calculate disease severity, the following formula was applied: PASI = 0.1(Eh + Ih + Dh)Ah + 0.3(Et + It + Dt)At +0.2(Eu + Iu + Du)Au + 0.4(El + Il + Dl)Al. PASI scores rise or fall in units of tenths (0.1) and range from 0.0 to 72.0. A score of 0.0 indicates absence of lesions, while a score of 72.0 represents the malignant form of the disease (erythrodermia). Body surface area involvement of over 10% or a PASI score greater than 10.0–12.0 is used as a criterion for severe disease. Percent PASI reduction was calculated as follows: (PASI at base line—PASI at each visit)/PASI at baseline x 100.Baseline PASI compared with post-treatment values were: PASI 100, 23%; PASI 75, 45%; PASI 50, 13%; PASI 10, 9%; <PASI 10, 3% while 7% quit treatment for economic reasons. Of the 648 subjects (23%) who experienced total remission of lesions, 188 (29%) had relapses of their disease after 15.4 months. The PASI values at the time of the first relapse were 7.7 units, one-third of the PASI value (21.0 units) recorded before any treatment. The new remission occurred with 7.1 doses of AS100-1VT after 5.8 weeks, a shorter time period than initially observed in the first treatment cycle for clinical remission of lesions. In the relapsing group, 161 of the 188 subjects (85.6%) experienced new remission of lesions after six to seven doses of AS100-1VT [56].

There were no serious adverse events attributed to the vaccine. Of the 2,770 subjects treated in the open label psoriasis study, a random group of 108 subjects was selected for antibody screening. The test group included a positive control group with active CL, a negative control group before treatment and subgroups with one to six doses of the immunotherapeutic agent. Subjects received 500 µg/dose of AS100-1VT. The concurrent negative control group (n = 36) consisted of psoriatic subjects, prior to any AS100-1VT treatment, with no previous history of *Leishmania* infection, no prior exposure to AS100-1VT and with a negative delayed type hypersensitivity (DTH) to *Leishmania* antigens. All subgroups with one to six doses of AS100-1VT in the treatment group had the same results as the negative control group, exhibiting antibody values between 30 and 87 ng/ml; well below 100 ng/ml, the cutoff value to consider a reaction as

positive, 3 standard deviations above baseline. It is also interesting to note that after four doses of AS100-1VT, all subjects had undetectable levels of antibodies (ELISA), but a positive DTH cellular response to AS100-1VT after intradermic reaction (IDR) ≥10 mm in diameter with *Leishmania* antigens. These results suggest that the AS100-1VT vaccine does not induce significant humoral immunity but a strong cellular immune response [56].

Approximately 2,289 subjects (83%) experienced at least one adverse event (AE), injection site related, and included the following: pain 43%, nodule formation 23%, heat 21% and erythema 14%, were relatively short lived, lasting between 24 to 72 h. The types of systemic AE were as follows: fever 18%, general discomfort 12%, a flulike syndrome 11%, pruritus 8%, sleepiness 8%, accidental injury 8%, cough 6%, dizziness 6%. There were no age or gender differences observed for AE, and no deaths occurred during the study. All adverse events resolved without intervention, usually within 24–72 hrs [56].

4.5 Purification of Effective Factor in Amastigotes Inducing Remission of Psoriasis

To determine the effective factor, a single blind trial with four monovalent second generation vaccines (AS100-2VT) were performed, with four treatment groups, one for each AS100-2 vaccine: AS100-2amazonensis, AS100-2brasiliensis, AS100 2chagasi, and AS1002venezuelensis. The trial included 26 subjects, 58% females, average 43.8±16.4 years old, and age range 8–76 years, initial PASI 10.2±6.6 units, time with psoriasis 12.2±13.1 years. Treatment subjects received 500 µg/dose injections of AS100-2 and control subjects received 500 µg/dose of AS100-1VT parasites from *La, Lv, Lb* and *Lch*, each species at a ratio 1:1:1:1 were injected at 500 µg/dose in 0.5 µl intramuscularly in the deltoid. The results achieved with monovalent AS100-2VT produced reductions in psoriatic lesions similar to those induced by polyvalent AS100-1VT. AS100-2VT vaccines were further purified, resulting in seven Diethyl Ethyl Amino Ethyl (DEAE) chromatography fractions (AS200) per species. AS100-2VT, and AS200 final product, gave the following results for LPG and endotoxin: LPG: 10 ng/ml or less; Endotoxin: <50 EU/ml but >25 EU/ml. Parasitic DNA was absent in all products and Bovine Serum Albumin (BSA) was between 12.5 and 25.0 ng/ml, basically within the same range, as previously published AS100-1VT values. No carbohydrates were found in AS100-1VT, AS100-2VT or AS200 *Lb* fractions by HPLC analysis or staining of gels with PAS-SCHIFF [20]. Subsequently, a single-blind trial in 55 subjects treated with a third generation vaccine AS200 prepared with seven DEAE chromatography fractions from Lb was performed. The AS200 study was a single-blind, AS100-1VT controlled trial, with a seven arm treatment group, one for each AS200 (*Lb*) vaccine. The AS200 trial included 53 subjects, 62% females average 40.6±18.6 years old, age range 7–78 years of age, initial PASI 26.4±19.2, time with psoriasis 15.0±12 years. The treatment subjects received four 200 µg/dose injections of AS200 (*Lb*) and control subjects received four 500 µg/dose of AS100-1VT. All AS200 (*Lb*) vaccines induced PASI reductions in the same range as the active control. Protein (DEAE) fractions 2, 3, 4, and 5 had similar PASI reduction values and induced the highest *in vitro* lymphocyte stimulation index (SI) values in PBMC from post-treatment PASI 100% reduction subjects, with no statistical differences among them. The same fractions, from Lb and Lch *s*pecies, also yielded the highest IDR diameter in DTH screenings. All *Leishmania* DEAE fractions stimulated lymphocytes from PBMC from patients *in vitro*, after AS100-1VT vaccine, none were immunosuppressive, contrary to all treatment in the market today [20].

4.6 Heat Shock Proteins and Psoriasis

Long-term HSP confrontation of the immune system similar in the host and invaders may convert the immune response against these host antigens and promote and/or decrease autoimmune diseases including psoriasis [57,58]. There is evidence that recognition of self-HSP60 can have beneficial effects in arthritis and may offer new strategies for improved control measures in the inflammatory processes by administration of peptides cross-reactive to self-determinants [59]. HSP60, HSP70, Gp96 function as host derived ligands for toll like receptors (TLR2), and have been described to play a role in the pathogenesis of RA and psoriasis [60] *Leishmania* antigens are produced after a heat shock in promastigotes that become amastigotes in a liquid axenically culture medium without mammalian cells present [61]. The molecular weight of AS200 is similar to the range of most HSP host ligands (50–70 kDa) and could be inhibiting the symptoms of psoriasis, psoriatic arthritis and collagen induced arthritis (CIA) in mice by competing with peptides in the respective receptors [62].

4.7 Amastigotes AS100-1 VT Vaccine Induced Remission of Psoriasis in HIV+ Patients

Two male subjects, both HIV+, 36 and 41 years old respectively, with plaque psoriasis for 4 years in one subject and 7 months in the other were treated with AS100-1VT. Both patients asked to be incorporated to the 2,770 subjects in the study group with psoriasis. Both subjects had no familial history of psoriasis, and were treated with AS100-1VT concurrently with their previously prescribed retroviral treatments. The AS100-1VT treatment consisted of 500 µg/dose, injected in the deltoid area, every 2 weeks. Baseline PASI for one subject was 26.4 units, while the other subject presented with a baseline PASI of 32.4 units. Both subjects showed clinical remission of psoriasis after treatment with AS100-1VT. The hematologic values with respect to total lymphocytes (CD3, CD4, and CD8 cells) were lower than the values routinely found in healthy subjects. One subject had a relapse after the ninth dose and subsequently received a second course of treatment 3 weeks later. The second course resulted in clinical remission of the psoriatic lesions. Neither of these two subjects presented local adverse events nor did they present any systemic adverse events after vaccination [20]. This points out that AS100-1 VT vaccine could have an effect inducing clinical remission of psoriasis even in a person with immunosuppression by AIDS. More trials on HIV+ patients will be require to confirm the findings on this two volunteers.

4.8 Purified Fractions Induced Remission of Collagen Induced Arthritis

AS200 *Leishmania* antigenic fractions induced linear DTH reactions in guinea pigs over a 1-40 µg dose range, which subsequently was used as a potency assay for AS100-1VT vaccine. Interestingly, RA, another autoimmune disease, shares several similarities with psoriasis and PsA. While some diseases lack acceptable animal models for adequate study, this is not the case with RA. CIA is an experimental animal model that has been used to dissect the pathogenesis of human RA. The model is dependent on activated T cells, is associated with both, cell mediated and humoral immunity to collagen and can be induced upon immunization with heterologous collagen II (CII) or by monoclonal antibodies to CII combined with LPS in DBA/1 mice. When a DBA-1 mouse CIA model was used to compare AS200 treatment against: a polyvalent vaccine (AS100-1VT), a monovalent vaccine (AS100-2) and placebo, the AS200 treated mice had the least amount of forepaw inflammation and the lowest mean arthritis scores [62,63].

4.9 Lymphocyte Subsets in Psoriatic Patients before and After AS1001-VT Treatment

PBMC collected from psoriatic subjects prior to treatment and post-treatment with AS100-1VT vaccine were analyzed by flow cytometry. LS varied with PASI range (1-10, 11-20 and 21-72). Pretreatment absolute values of gated LS were as follows: CD4+CD8-, CD3+CD8-, CD8+CD3+, CD8+CD4- and CD8+HLA- decreased in PBMC as PASI increased, suggesting migration from the blood to the skin. Contrary to the previous finding, the following LS, CD8+HLA+, HLA+CD8-, CD8+CD4+, CD19, and membrane surface immunoglobulin IgA+, IgD+ and IgM+ increased in PBMC as PASI increased, suggesting activation and proliferation by unknown antigens in the skin lesions. After treatment with seven doses of AS100-1VT vaccine, the following LS, CD3+CD8-, CD8+CD3-, HLA+CD8-, CD8+HLA+ and CD4+CD8-, increased as PASI returns to normal values and psoriatic plaques disappeared, while CD8+CD3+, CD8+HLA-, CD19 and CD8+CD4+ decreased in PBMC suggesting lower sensitization in skin. Lymphocyte trafficking from blood to skin decreased significantly, stopping the vicious cycle as psoriasis lesions disappeared [64].

Previously we demonstrated that *Leishmania* antigens induced T cell proliferation and absence of immunosuppression after stimulating PBMC from psoriatic patients with amastigote fractions. DTH positive reactions were found with isolated amastigotes antigens in humans in vivo, after treatment with AS100-1VT polyvalent vaccine [20]. These facts of *in vivo* and *in vitro* T cells stimulation suggest that variations in blood LS, before and after treatment in psoriatic subjects, is a function of lymphocyte trafficking from blood to skin and vice versa, as well as T cell activation in skin plaques, not the killing of T cells as has been described with current treatments used in psoriatic patients.

5. PSORIATIC ARTHRITIS CLINICAL FEATURES

All studies of PsA use the criteria by Moll and Wright in their classic paper published in 1973 [65] summarized as follows: A-Presence of psoriasis, B-Inflammatory arthritis and C-

Negative test for rheumatoid factor. The PsA subgroups described with these criteria were: 1-Distal interphalangeal (DIP) joint disease (5%); 2-Asymmetrical oligoarthritis (70%); 3-Polyarthritis (15%); 4-Spondylitis (5%); 5-Arthritis mutilans (5%). Gladman expanded the five sub-groups to seven: Distal disease (DIP only affected), oligoarthritis (<4 joints), polyarthritis, spondylitis only, distal disease plus spondylitis, oligoarthritis plus spondylitis and polyarthritis plus spondylitis [66]. All these criteria have been applied to our patients with PsA. The genes involved, in PsA are HLA genes of class I MHC alleles, on the HLA-B and HLA-C loci. Psoriasis is linked to HLA-Cw6 allele. Twenty percent of PsA patients with peripheral joint involvement displayed HLA-B27, a value that climbs to 70% in patients with PsA type spine involvement [67]. In psoriasis, the association has been primarily with class I antigens: HLA-B13, HLA-B17, HLA-Cw6, and HLA-Cw7, the strongest association being with HLA-Cw6 [68]. The results of multiple well powered genome wide association studies have identified several loci outside the MHC region associated with psoriasis risk, including three genes involved in IL-23 signaling (IL-23R, IL-23A, IL-12B), two genes that regulate nuclear factor-κB signaling (TNIP1, TNFAIP3), and two genes involved in the modulation of T-helper type 2 immune responses (IL-4, IL-13) [69].

5.1 Psoriatic Arthritis Pathogenesis

In patients with PsA, carotid wave pulse velocity, a measure of arterial stiffness, was significantly higher. Patients with psoriasis were found to have increased coronary artery calcification in direct imaging study compared to controls. Two case control studies also demonstrated that patients with PsA had a higher prevalence of subclinical atherosclerosis as measured by arterial intima-media wall thickness (IMT) and endothelial dysfunction without overt CV disease. In patients without clinical CV disease 35% had increased IMT despite having low CV risk. 102 patients with PsA had a higher prevalence of type II diabetes mellitus and hypertension, and an increased prevalence of HDL cholesterol, apolipoprotein A1 levels, lower total cholesterol and LDL cholesterol levels, and lower total cholesterol to HDL cholesterol ratio [70]. Both the innate immune system and T helper-1 lymphocytes appear to be involved in atherogenesis. This is similar to the pattern of immune mediated inflammation in psoriasis and PsA. In all patients CV disease was defined as a history of myocardial infarct (MI), stroke and/or transient ischemic attack verified by written documentation of the event. The prevalence of CV disease was 10% in patients with PsA compared with 12% in patients with RA [71].

5.2 Lymphocyte Subsets in Psoriatic Arthritis before and after AS1001-VT Treatment

As more skin disease is present in PsA patients, more inflammation is found in the joints, suggesting a link between skin and joint inflammatory processes; since both were exacerbated in the PASI 100 and PASI 75 groups and also needed higher number of doses to achieve a lower arthritis score, tender joints and nail changes values [72]. Absolute values of gated LS before treatment decreased in this order: CD8+HLA-,CD8+HLA+,CD4+,CD8+CD3+,CD8+CD3 in PBMC as PASI increased, suggesting migration of CD8+ cells from the blood to the joints and skin. Contrary to the previous finding, LS:CD8+CD4-,CD3+CD8-,HLA+CD8-,CD19+, CD8+CD4+,IgA+,IgD+,IgM+, IgE+ and IgG+ increased in PBMC as PASI increased suggesting activation and proliferation by unknown antigens and subsequent migration to the blood. The LS quantification in this group of PsA patients only (n=508) were different [72] to the LS quantified in the psoriasis skin disease trial (n=2770) [64], since in PsA the majority belonged to the CD8+ phenotype, a T cell key in the PsA inflammatory process as described by many authors. In PsA patients there is also evidence of T cell recirculation before treatment and a vicious cycle with T and B cells migrating between blood and skin and joints. After treatment with nine doses of AS100-1VT vaccine, a dramatic decrease in LS belonging to T and B cells, in PBMC was observed, as PASI, AS, tender joint counts, and nail changes returns to normal values and the vicious cycle disappeared [72].

5.3 Inflammatory Markers in Psoriasis before and after AS100-1VT Treatment

AS100-1VT had a cellular not humoral immune response as supported by the DTH and ELISA results in humans and guinea pigs. All psoriatic patients were DTH positive after the third vaccination with AS100-1VT, but no antibodies were detected in serum from these volunteers with up to 6 doses of vaccine [56,62]. This suggests that the immunological response to AS100-1VT after TLCK treatment and NP-40 extraction was mediated by T-regulatory cells,

with no antibody production, a novel mechanism that may play a role in decreasing inflammation in psoriatic skin. Amastigote peptides may induce Th3 regulatory T cells producing IL-10 that inhibits Th1 and Th2 cell cytokine production and induced peripheral cell tolerance.

Inflammatory markers C-reactive protein (CRP) and complement 5a (C5a) assayed in two PsA patients decreased significantly in serum after treatment with 6 doses of AS200 DEAE fractions 3 + 4 Leishmania amastigotes antigens at 300 µg/dose (Fig. 5). The *Leishmania* antigens decreased markedly the TNFα concentration in supernatants from PBMC in both patients and controls.

PBMC of patients and controls were stimulated with concanavalin A (ConA); ConA+AS100-2 *L(L) chagasi* antigens and compared to no treatment as control. The *Leishmania* antigens decreased markedly the TNFα concentration in supernatants from PBMC in both patients and controls (Fig. 6). In mice ConA induced hepatitis, injection of 50 µg AS100-2 *L(L) chagasi* antigens subcutaneously (SC) decreased serum TNFα as compared to placebo (PBS) in 8 hours of observation (Fig. 7). Serum IL-1β, 8 hours after SC injection of AS200 *L(V) brasiliensis*+RH and AS100-1+RH in mice, also decreased significantly as compared to placebo and in a range similar to the positive control dexamethasone (Fig. 8). AS100-2 *L(L) chagasi* antigens decreased proliferation of cutaneous T cell lymphoma in vitro in a dose-response relationship (Fig. 9).

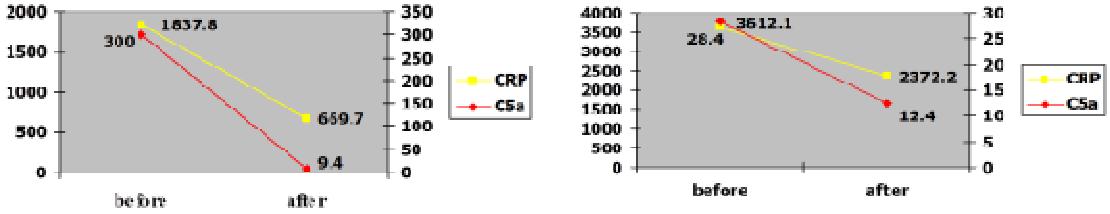

Fig. 5. Inflammatory markers CRP and complement C5A in sera from Psoriatic patients with 6 doses of AS200 F3+F4 at 300 µg/dose before and after treatments. Both patients had PASI reduction 61.6% and 66.4% respectively and biopsies with excellent improvement evidenced by decrease of epidermal layer and absence of inflammatory cells in epidermis and dermis in comparison to placebo (Rehydragel). Adapted from reference 21

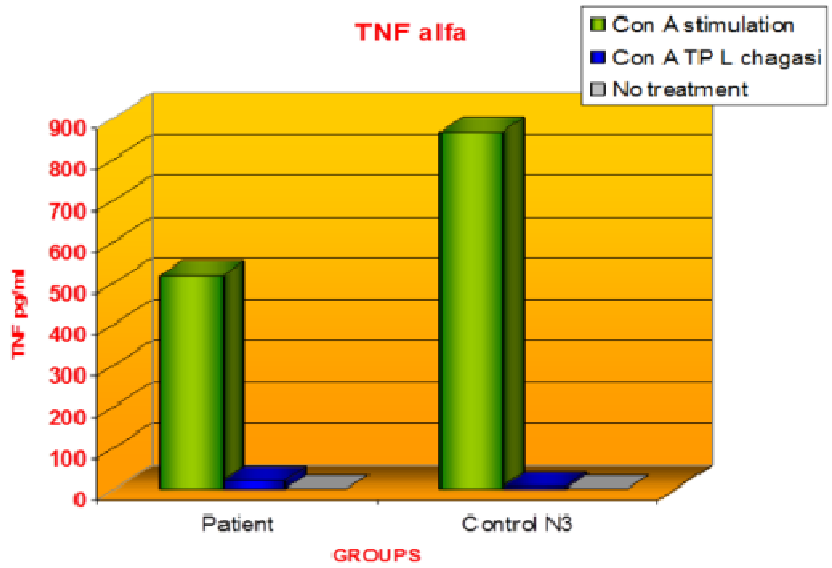

Fig. 6. PBMC of patients and controls after stimulation with concanavalin A (ConA), ConA +AS100-2 *L(L)chagasi* antigens or no treatment 3 patients and 3 controls per group. Adapted from reference 21

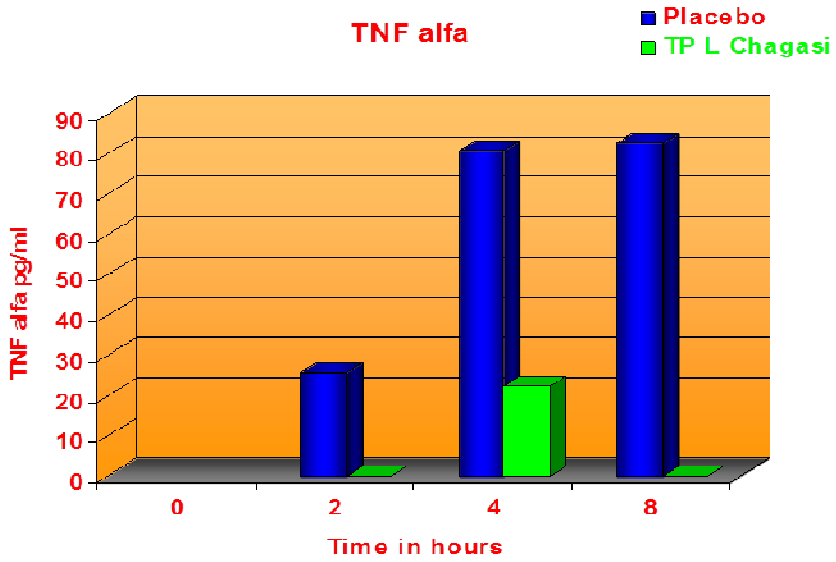

Fig. 7. Serum TNFα in ConA induced hepatitis in mice (n=3 per time group). After SC injection of 50 µg AS100-2 *L(L) chagasi* antigens, TNFα decreased significantly as compared to placebo (PBS) at 2, 4 and 8 hours observation period. STDEV <5% of average. Adapted from reference 21

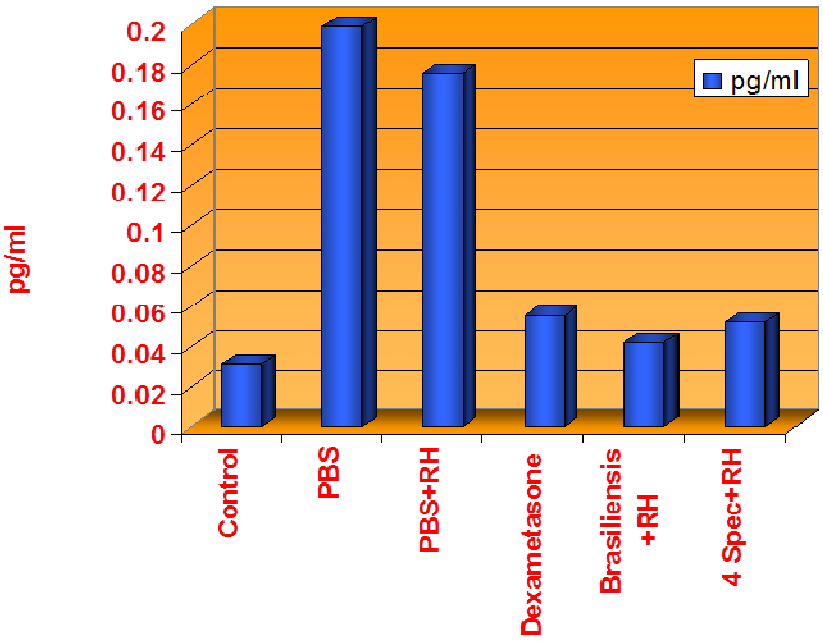

Fig. 8. Serum IL-1β in ConA induced hepatitis in mice (n=3 per group). IL-1β was determined after 8 hours of SC injection of the following products: 1- 50 µl placebo (PBS); 2- 50 µl PBS + Rehydragel (RH); 3- 50 µg/mice *L(V)brasiliensis* AS200 fraction 3 and 4 + RH; 4- AS100-1(4 species VT vaccine)+RH; 5- 1 mg/Kg/mice Dexamethasone. Control normal mice, received no treatment. IL-1β decrease significantly after treatment with *L(V)brasiliensis* AS200 antigens 3 and 4, or polyvalent AS100-1 VT vaccine, similar to dexamethasone as compared to placebos 1 and 2 treatment n= 3 per group. Adapted from reference 21

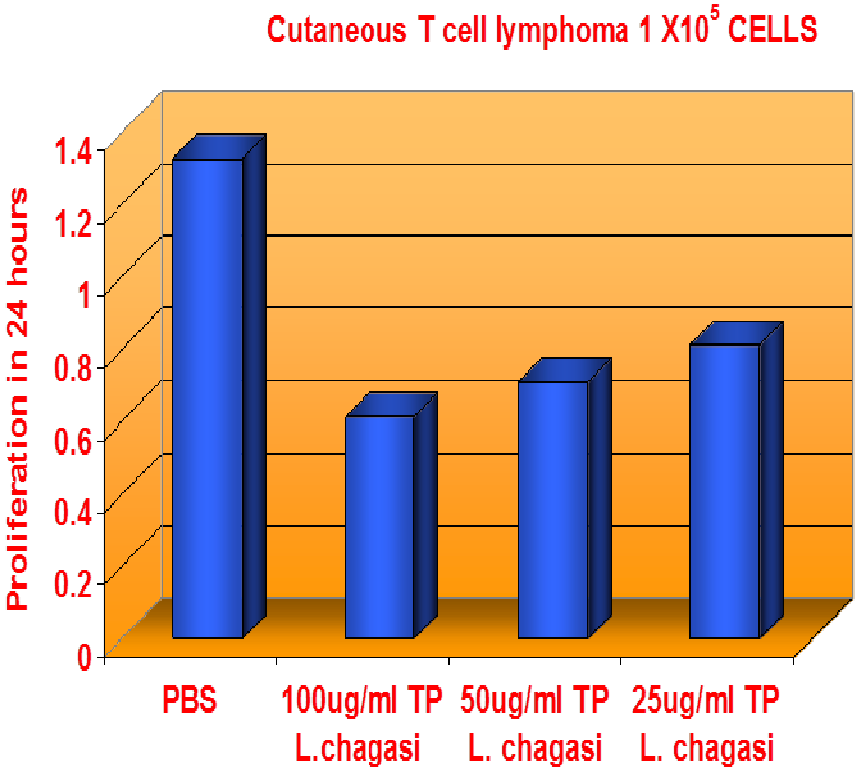

Fig. 9. In vitro proliferation of cutaneous T cell lymphoma cells at different concentrations of AS100-2 *(L) chagasi* leishmania antigens as compared to PBS after 24 hours of culture. Values are average of three different experiments. STDEV < 5% of average. Adapted from reference 21

6. HISTOPATHOLOGY IN PSORIATIC PATIENTS

Placebo (Aluminum hydroxide, Rehydragel) injections in biopsies from patients showed increase in epidermal and dermal inflammatory cells. Epidermis size and keratin staining increased markedly, the latter a sign of cell proliferation. Patients had with very low PASI reduction, with DTH and ELISA negatives as expected (Fig. 10).

Injection of placebo+150 µg of AS200 vaccine exhibited a different picture. Inflammatory infiltrates decreased, cellular immunity as measured by DTH was positive, and % reduction in PASI values reached 55% by effect of the vaccine (Fig. 11).

Injection of placebo+300 µg of AS200 vaccine showed a greater reduction of inflammatory infiltrates, cellular immunity as measured by DTH was positive, as well as humoral immunity as measured by ELISA. % reduction in PASI values reached 58% by effect of the vaccine (Fig. 12).

Injection of placebo+500 µg of AS200 had the greatest decrease in CD3+ T cells infiltrates and keratin staining with 61% PASI reduction in psoriatic patients (Fig. 13).

Clinical results after injection of polyvalent AS100-1VT vaccine exhibited excellent PASI reductions with final PASI 0.5 in palm plantar psoriasis (Fig. 14); zero in body +palm-plantar psoriasis (Fig. 15), 1.8 in plaque psoriasis (Fig. 16) and zero in plaque +gutatta psoriasis with no relapses (Fig. 17). Comparison of PASI values between placebo and placebo+AS200 values had $P<0.001$ by Student T tests.

6.1 Biopsies of Psoriatic Patients after Placebo LS: Before Treatment

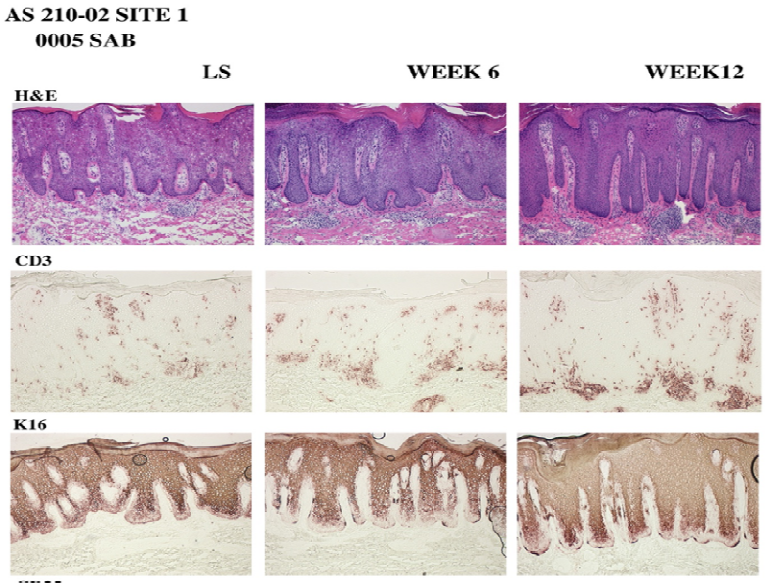

Fig. 10. Epidermis and inflammatory infiltration increased after Hematoxyline and Eosin staining as well as CD3 T cells and keratin staining after 6 doses of placebo one every 2 weeks. Size epidermis /base line: -22.0 units. DTH: negative. ELISA: negative. % PASI reduction: 26.4. Final PASI: 18.9%. DTH: delayed type hypersensitivity reaction. ELISA: enzyme linked immunoabsorbent assay. PASI: Psoriasis Area and Severity index

6.2 Biopsies of Psoriatic Patients after AS200 Treatment LS: Before Treatment

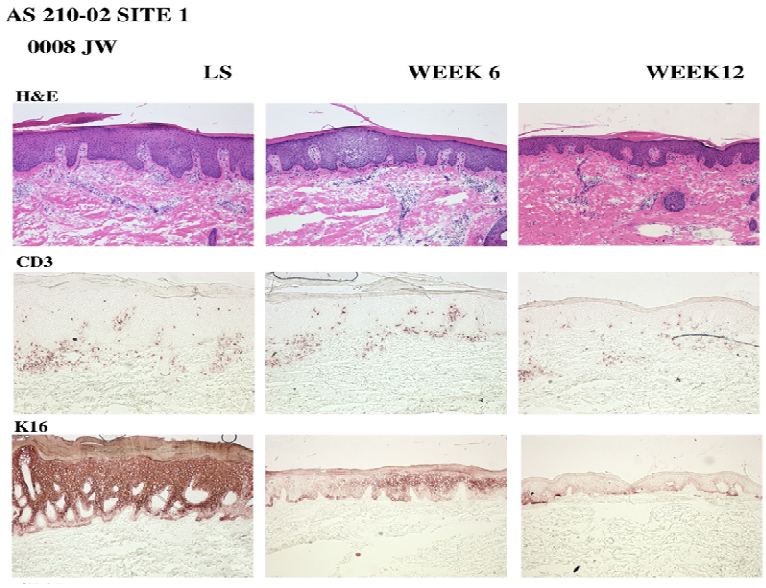

Fig. 11. Epidermis and inflammatory infiltration decreased markedly after Hematoxyline and Eosin staining as well as CD3 T cells and keratin staining after 6 doses of 150 µg one dose every 2 weeks. % decrease size epidermis /base line: 63.6 units. DTH: positive. ELISA: negative. % PASI reduction: 55.1. Final PASI: 47.4 % . DTH: delayed type hypersensitivity reaction. ELISA: enzyme linked immunoabsorbent assay. PASI: Psoriasis Area and Severity index

6.3 Biopsies of Psoriatic Patients after AS200 Treatment LS: Before Treatment

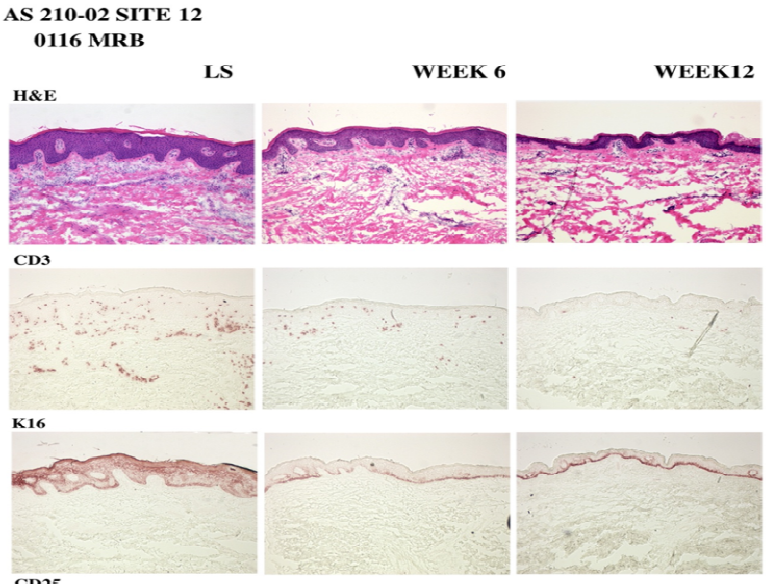

Fig. 12. Epidermis and inflammatory infiltration decreased markedly after Hematoxyline and Eosin staining as well as CD3 T cells and keratin staining after 6 doses of 300 µg one dose every 2 weeks. % decrease size epidermis /base line: 52.1 units. DTH: positive. ELISA: positive. % PASI reduction: 58.1. Final PASI: 27.4 %. DTH: delayed type hypersensitivity reaction. ELISA: enzyme linked immunoabsorbent assay. PASI: Psoriasis Area and Severity index

6.4 Biopsies of Psoriatic Patients after AS200 Treatment LS: Before Treatment

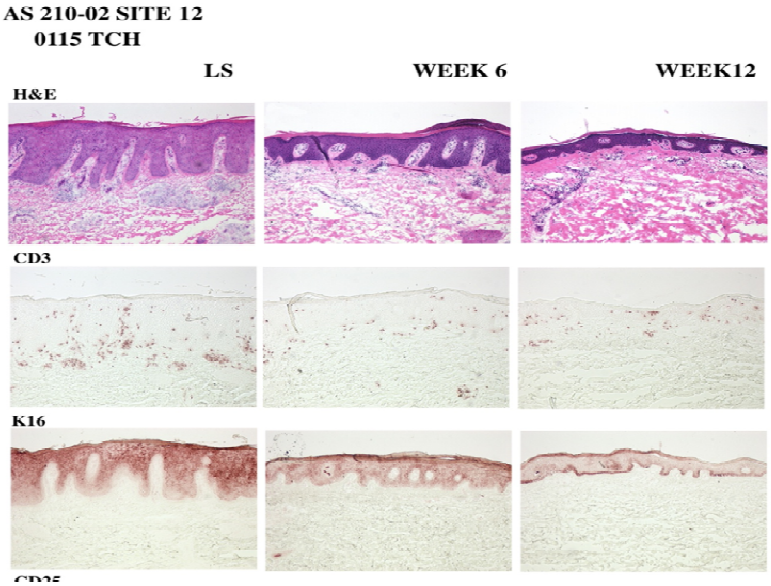

Fig. 13. Epidermis and inflammatory infiltration decreased markedly after Hematoxyline and Eosin staining as well as CD3 T cells and keratin staining after 6 doses of 500 µg one dose every 2 weeks. % decrease size epidermis /base line: 66.3 units. DTH: negative. ELISA: negative. % PASI reduction: 61.4. Final PASI: 10.8 % DTH: delayed type hypersensitivity reaction. ELISA: enzyme linked immunoabsorbent assay. PASI: Psoriasis Area and Severity index

7. CLINICAL ANALYSIS IN PSORIATIC PATIENTS BEFORE AND AFTER AS100 VT VACCINE

Fig. 14. Male, 41 years old, 7 years with plaques. Previous treatment: topical steroids. Initial PASI: 10.5 (04-02-1997). Final PASI: 0.5 (03-12-1998) Doses/periodicity: 8, 30-60 days. Time remission: 11 months. PASI at relapse: 2.8(09-07-1998). Two months later, total cure without any treatment

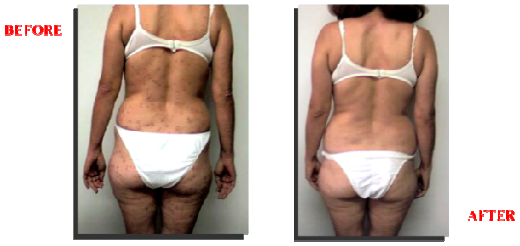

Fig. 15. Female, 42 years old. Two years with Gutatta. Previous treatment: Intradermic + topic steroids. Triggers: Ethanol, stress, menstruation, tobacco. Initial PASI: 10.1(03-19-1999). Final PASI: 0 (08-11-1999).Doses/periodicity: 11/ every 15 days. Time remission: 5 months. PASI at relapse: 0.07. (01-12-2000). Time for relapse: 17 months. New doses/periodicity: 5/30 days. New PASI: 0 (06-15-2000). Time for new remission 5 months. Relatives: Mother, cousin, sister

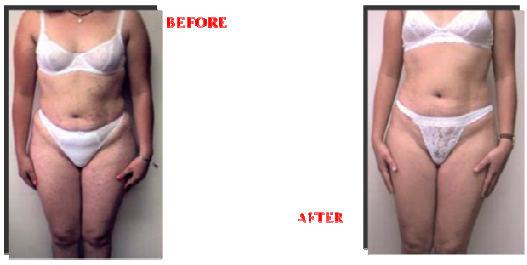

Fig. 16. Female, 66 years old. Forty years with plaques. Previous treatment: Topic steroids, methotrexate, Vitamin A derivatives, coal tar. Triggers: stress. Initial PASI: 25.6 (06-07-1999). Final PASI: 1.8 (11-11-1999). Doses/periodicity: 12/ every 15 days. Time remission: 5 months. Relapses: none. Relatives: mother

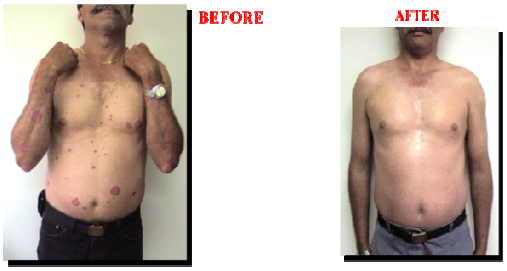

Fig. 17. Male, 42 years old. 21 years with plaques + Gutatta. Previous treatment: Topic steroids, methotrexate, Vitamin A derivatives, UVB. Initial PASI: 25(04-09-1997). Final PASI: 0 (05-06-1999).Doses/periodicity: 60/ every 10 days. Time remission: 24 months. Relapses: none

8. CONCLUSION

Amastigotes from four *Leishmania* species (VT, AS100-1VT vaccine), axenically cultured induced clinical remission of cutaneous leishmaniasis and protected volunteers in six years of follow up. Humoral immunity decreased after clinical remission of lesions. Intracellular parasitism is due to normal antibodies recognizing parasite antigens post-inoculation by vector. VT vaccine induced mainly cellular immunity, measured by DTH intradermic reaction, post remission of lesions. No patients had relapses or secondary infections after VT treatment in primary infections. VT vaccine induced clinical remission of all forms of Psoriasis, Psoriatic arthritis, and Rheumatoid arthritis in human volunteers a serendipity finding without any serious adverse events. The vaccines are not immunosuppressors, but immunomodulators whose effect is due to decrease in inflammatory cytokines. The pool of potential factors was down to four protein (DEAE) fractions (AS200) suppressing psoriasis by stimulating lymphocytes, a mechanism of action contrary to most drug products used today. Collagen induced arthritis in mice also decreased with AS200. VT vaccine inhibited proliferation of cutaneous T cell lymphoma *in vitro* which open new roads for the application of this vaccine to wide spectra of diseases. Blood lymphocyte subsets analyzed by flow cytometry in psoriasis and Psoriatic arthritis patients had different values as PASI changed. Absolute values of some gated LS decreased in PBMC as PASI increased, suggesting migration from the blood to the skin while others increased suggesting activation and proliferation by unknown antigens. After treatment with VT, LS

increased to normal values as PASI returns to normal, suggesting lower sensitization and prevention of lymphocyte trafficking from blood to skin as psoriasis lesions disappeared. Finally, many doors have been opened with the serendipity discovery of *Leishmania* amastigote antigens inducing clinical remission of cutaneous leishmaniasis, psoriasis, psoriatic arthritis and rheumatoid arthritis as systemic diseases, which no doubt will provide new roads and answers not solved yet. Future research and clinical efforts will illuminate many fundamental problems, encountered in those terrible illnesses affecting human beings in all countries on earth.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHIC COMMISSION

The clinical investigations were conducted in accordance with the Declaration of Helsinki. The Ethic Commission of the National Academy of Medicine of Venezuela and also the Ethic commission of the "National Institute of Scientific Investigations (IVIC)" approved the protocols for the field trials for leishmaniasis as well as, the trials for Psoriasis. Dr. Blas Bruni Celli was appointed, trial monitor by the National Academy of Medicine of Venezuela, and oversaw all subsequent follow-up work on the trials. All volunteers signed an informed consent authorizing treatment. Phase I and II trials with AS200, in USA were also approved by Western Institutional Review Board (WIRB) and the FDA.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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