IMMUNOPATHOLOGY OF AUTOLEUKOCYTIC THERAPY OF CUTANEOUS LEISHMANIASIS

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This is a report of immunopathological study of our previous work on autobuffy-coat therapy of dry-type cutaneous leishmaniasis, which focused on cytological activation of macrophages to eradicate engulfed Leishman bodies by the synergistic effects of lymphocytes, neutrophils, and inactive monocytes.

We, again, present a case report of skin biopsies which were stained immunohistochemically before and during autobuffy-coat therapy.

The results revealed increased CD4 and CD8 lymphocytes, CD1a Langerhans cells, interferon gamma, and TNF-alpha-producing cells during therapy, but IL-10, IL-4, and TGF-beta-positive immunostained cells were decreased.

We conclude that this kind of therapy induces a Th1-like and suppresses a Th2-like response in active lesions of dry-type cutaneous leishmaniasis accompanied by overactivity of the neutrophils and activated histiocytes to eradicate the engulfed Leishmania amastigotes.

Keywords: Autoleukocytic therapy • cutaneous leishmaniasis • cytokines

Introduction

Survival and replication of Leishmania in its safe target macrophages are due to several evolutionary mechanisms to modulate host signal pathways of its eradication. Different therapeutic regimens—local and systemic—have been used to activate macrophages to eradicate the engulfed Leishman bodies. Th1-like response activates this parasitocidal effect and Th2-like response decreases the intracellular eradication of the parasite.

Autologous blood components have been used in leukemic patients undergoing bone marrow transplantation and autologous T cells have been used as components of photoinmunotherapy for cutaneous lymphoma.

Continuing our previous work and the first-time case-reports of autobuffy-coat therapy in dry-type cutaneous leishmaniasis (DLCL), we present another case report, which focuses on different immunophenotyping of immune effector cells as well as cytokines profiles of Th1 and Th2 patterns during autoleukocytic therapy in skin biopsies.

Case Report

An 18-year-old male suffering from a raised nonulcerated nodule (measuring 2.5 × 1.5 × 1.5 cm) of the frontal area with a six-month duration presented to dermatology clinic. Informed consent of the patient was obtained. Diagnosis of cutaneous leishmaniasis was based on clinical picture and characteristic features seen on skin scraping preparation and histopathological findings on skin biopsy. A 10-mL sample of peripheral blood was
drawn into a heparinized tube, centrifuged at 37°C for 15 minutes at 2,500 rpm. Plasma was decanted and the buffy-coat layer was aspirated into sterile insulin syringe. A sample of 0.25 – 0.5 mL of buffy-coat suspension containing 200,000 to 400,000 cells was injected intraleSIONally by the dermatologist using multiple injection sites. This therapy was repeated at one-week interval injections for three times. At the beginning of the fourth week, the patient received intraleSIONal glucantime every other day, for one week. The lesions were examined clinically for size, induration, and softness. In addition, punch biopsies (2 – 4 mm) were taken from the lesion before therapy (baseline), after two injections (2 weeks), and finally after 4 weeks. Half of the skin biopsy was fixed in 10% formalin, embedded in paraffin, and finally stained with hematoxylin and eosin. The other half was embedded in OCT and snap-frozen in liquid nitrogen. Frozen sections were cut and used for immunoperoxidase staining.

Immunoperoxidase staining was CD4 (Dako M0716, dilution factor = df; 1:10), CD8 (Dako M0707, df; 1:100), CD1a (Dako M0721, df; 1:100), CD14 (Dako M0852, 1:10), HLA-Dr (Dako M0704, 1:50), CD19 (Dako M0740, 1:25), CD56 (Dako M0852, 1:10), INF-gamma (Genzyme, 1598–00, 1:20), TNF alpha (R&D, MAB 219, 1:25), IL-12 (R&D, MAB219, 1:25), IL-4 (Genzyme, 1842–01, 1:10), IL-10 (R&D, MAB215, 1:20) and negative control Mouse Ig1 (Dako X 0931, 1:10).

Four individual pathologists scored the immunostained slides independently. Percentages of positive immunostained cells among 200 – 400 cell populations in their five most crowded areas (0.0009 mm²) were counted with high-power fields (×40) and the percentage of positive cells was noticed in flow-charts. The data were expressed by mean ± standard error of mean (sr). Th1 response was calculated by the mean of the summation of positive cells of INF-gamma, TNF-alpha, and IL-12; and Th2 response as the mean of summation of positive cells of IL-4, IL-10, and TGF-beta. The lesion showed a beneficial subjective and objective clinical responsive to the therapy.

Skin scraping slides showed infiltration of lymphocytes, inactive macrophages, and neutrophils between the anergic collections of the histiocytes which engulfed by Leishmania amastigotes (Figure 1). The number of Leishmania amastigotes was dramatically decreased with some degenerative changes of activated macrophages (Figure 2).

By looking at the graphs of the immunophenotyping pattern of positive immunostained cells during therapy and comparing them with baseline data, we concluded that CD4+ and CD8+ lymphocytes were increased to 3 – 4 times more than before therapy (Figure 3). CD1a was 1.5 times increased. INF-gamma and TNF-alpha were increased to 2 times the level prior to therapy. IL-12 was increased as well. IL-4, IL-10, and TGF-beta were decreased to one-half. CD19, and CD56 were negative both before and after therapy.

Discussion

Different local6, 7 and systemic8, 9 therapeutic regimens have been used to treat cutaneous leishmaniasis. On the other hand, mechanisms of parasitic control and evasion of immune response

Figure 1. Disruption of degenerated macrophage (arrow-head) and releasing of their engulfed Leishman bodies (short arrow). Peripheral rimming are neutrophils, lymphocytes, and macrophages (×1000, Giemsa stain).

Figure 2. Penetration of lymphocyte within ruptured parasitophorous vacuoles. Nuclear and cytoplasmic degeneration of macrophages are noticed (×1000, Giemsa stain).
are so complicated that the parasite knows how to retreat into “safe target cells”, actively suppress the synthesis of reactive oxygen or nitrogen intermediates, modulate the host cytokine response, inhibit antigen presentation, and T cell stimulation.\textsuperscript{10} Immunotherapy has been shown successful in the eradication of the disease. Sjolander et al showed that, in murine leishmaniasis, vaccination with plasmid DNA encoding the host protective \textit{L. major} parasite surface Ag-2 primed for an essentially exclusive Th1 response protected mice against \textit{L. major} infection.\textsuperscript{11} Handman et al mentioned that prophylactic DNA vaccination shifted the T cell-derived cytokine environment with an increase in the INF-gamma-producing Th1 type cells.\textsuperscript{12} Cabrera et al showed that by using live \textit{Mycobacterium bovis} BCG and heated killed \textit{L. amazonensis}, one could induce Th1 response and clinical cure mediated by INF-gamma.\textsuperscript{13}

According to our findings, autoleukocytic (buffy-coat) therapy increases the percentage of CD4\textsuperscript{+} and CD8\textsuperscript{+} lymphocytes and, from the point of view of their cytokine profile patterns, induce a more prominent Th1-like response than Th2-like response. Gaafar et al demonstrated a Th1 response in mild form and a Th2 response in nonhealed severe disease.\textsuperscript{14} Ajdary et al showed Th1 responses in active and recovered lesions and Th2 response in non-healed lesions.\textsuperscript{15} Furthermore, the presence of circulating effector immune cells in the peripheral blood of cured cutaneous leishmaniasis patients has been demonstrated.\textsuperscript{16, 17} In our series, CD4\textsuperscript{+} and CD8\textsuperscript{+} lymphocytes, Langerhans cells, INF-gamma, and TNF-alpha are increased, while \textit{Leishmania amastigotes}, macrophages, IL-4, IL-10, and transforming growth factor beta are decreased.

Salaiza-Suazo et al demonstrated that patients with diffuse cutaneous leishmaniasis caused by \textit{L. mexicana} have Th1 conditions if treated by two leishmanicidal drugs (pentamidine and allopurinol) in combination with recombinant INF-gamma. Additionally, parasites decreased dramatically, macrophages diminished concomitantly, while IL-12-producing Langerhans cells and INF-gamma-producing NK and CD8\textsuperscript{+} lymphocytes increased.\textsuperscript{18} Kolde et al revealed that interferon therapy in murine leishmaniasis could induce a Th1 response which activates macrophages to kill intracellular parasites and leads to a decline of less mature macrophages in the infiltrate.\textsuperscript{19}

In our series, the increased number of Langerhans cells and IL-12 cytokines concur with the findings of others. Li et al also documented that the treatment of nonhealing lesions in murine leishmaniasis through combination therapy of antimonial drugs with INF-gamma depends on the mechanisms of IL-12 and nitric oxide production.\textsuperscript{20} Kole et al showed that reducing parasite numbers, reduced IL-4 but increased IL-12 and inducible nitric oxide synthase are due to the synergistic effect of INF-gamma and mannosylated liposomal doxorubicin in the therapy of experimental visceral
leishmaniasis. Kropf et al showed that in murine leishmaniasis, although IL-4 polarized Th2 response, the induction of healing displayed a strongly elevated response to IL-12.

TGF-beta is a multipotential cytokine with diverse effects on immune cells, including the down-regulation of certain macrophages and the blockade of INF-gamma-induced macrophage activation. Li et al showed that using anti-TGF-beta treatment promotes rapid healing of murine leishmaniasis through enhancing in vivo nitric oxide production by activated macrophages.

Finally, our data show that autoleukocytic therapy is an effective and safe mode of therapy for active cutaneous leishmaniasis. Further investigations are mandatory to find out the possible effects of recruitment cytokines on immune effector cells.

Acknowledgment

We thank Mrs. Lis Christensen for excellent immunostaining of frozen specimen and Mrs. Azeri and Mrs. Gharaei for typing the manuscript. This research is funded through a grant from DANIDA and the support of the Clinical Microbiology Department, Copenhagen Medical School, Denmark.

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