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Translating immune cell cross-talk into a treatment opportunity for visceral leishmaniasis

“Results from both murine and human studies suggest the emergence of immunotherapy, involving different agonists and antagonists to the cytokines and cells involved in the course of disease progression, as a promising treatment option against visceral leishmaniasis.”

KEYWORDS: cytokine ■ fusion protein ■ immunotherapy ■ T cell

Visceral leishmaniasis (VL) or kala-azar, a disseminated infection of the lymphoreticular system caused by the complex family of protozoa *Leishmania*, is one of the most prevalent parasite-borne diseases worldwide and can result in death if not treated properly. The parasite resides primarily within macrophages of the liver, spleen and bone marrow, and the course of the disease indicates an underlying defect in the immune defense mechanism of the host [1].

In the absence of any vaccine, chemotherapy remains the most favored option for the control of VL. Until the year 2000, pentavalent antimonials were the mainstay of treatment for VL. However, the drug has developed resistance in parts of northern Bihar. This led to the introduction of amphotericin B (AmB) as an active antileishmanial agent, which has produced high cure rates (>95%) among VL patients in India. However, AmB, which requires long-term intravenous administration, is highly toxic and has frequent adverse effects, including infusion-related fever and chills, nephrotoxicity and hypokalemia. To overcome the drawbacks of AmB, less toxic and more effective lipid formulations of AmB have been developed that could significantly reduce organ toxicity as well as treatment duration. Most of these formulations are, however, exorbitantly costly. Recently, the WHO has reduced the price of one such formulation, AmBisome® (Astellas Pharma Inc., IL, USA), by a tenth for developing countries. On the other hand, miltefosine, the only known oral antileishmanial drug so far, has limited side effects with a consistently high cure rate. However, it is teratogenic and has a long half-life, which could encourage the development of clinical resistance [2].

The rational approach towards antileishmanial chemotherapy would, however, be to

potentiate the immune functioning of the host in addition to the killing of the parasite. A close look at the immune response during VL revealed IL-10 as the principal cytokine involved in disease pathogenesis whereas IFN- γ and IL-12 are major players for protection. All the conventional chemotherapeutic agents, in addition to their parasite clearance activity, are also capable of exerting immunomodulatory effects through a complex interplay of these cytokines. Pentavalent antimonials, along with its strong leishmanicidal activity through inhibitory effects on different biochemical processes of the parasite [3], could also suppress IL-10 in the course of treatment period [4]. AmB, as well as its liposomal formulations, not only downregulates IL-10, but also downregulates TGF- β , along with their ergosterol pore-based direct parasitocidal mechanism [4]. Miltefosine controls the parasite number directly through an apoptosis-like activity [5], and at the same time regulates the immune system through stimulatory effects on IFN- γ [MONDAL S, BHATTACHARYA P, MODAK D ET AL., UNPUBLISHED DATA]. However, as already discussed, monotherapies against VL are far from ideal. Moreover, these approaches have poor results in HIV/VL coinfecting cases [6]. This calls for combination therapy involving two different chemotherapeutic agents, with either an immunomodulator, or a recombinant cytokine/cytokine inhibitor. Administration of two antileishmanial agents of different chemical classes could not only reduce individual doses, toxicity and treatment duration, but also escalate their individual immunostimulatory effects. Combination therapy involving an immunomodulator or a recombinant cytokine/cytokine inhibitor is broadly classified as immunotherapy (also known as biotherapy). The first evidence of the use of immunotherapy

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against experimental VL came from the studies by Murray *et al.* [7] when an anti-IL-10 receptor monoclonal antibody (mAb) was reported to inflict parasite killing through an inducible nitric oxide synthase-dependent mechanism and increased IFN- γ expression. This approach was later confirmed by the observations of Gautam *et al.* [8]. The parasitocidal activity of anti-IL-10 receptor mAb was also evident in IFN- γ knockout mice, suggesting IL-10 blocking to be an important aspect of immunotherapy against VL [7]. Combination therapy with recombinant human IFN- γ and pentavalent antimonials could lead to a stronger parasitologic and clinical cure compared with the drug alone in VL patients from Brazil, Kenya and India [6]. Immunotherapy with recombinant human IFN- γ could also reduce the treatment duration of the conventional drugs. Efficacy of the chemotherapeutic agents could also be augmented when administered with different liposomal formulations. A combinatorial approach of stearylamine bearing positively charged phosphatidylcholine (SA-PC) along with sodium antimony gluconate could yield hepatic and splenic parasitocidal activities significantly higher than the drug itself [9]. Combination of SA-PC liposomes with AmB could not only clear parasites from both spleen and liver, but could also maintain the immunomodulatory effects of AmB by successfully down-regulating IL-10 secretion and enhancing IFN- γ , IL-12 and nitric oxide production [10]. Similar observations were also made when paromomycin (PM) was administered with SA-PC. However, in comparison with PM alone, SA-PC plus PM could significantly downregulate both IL-10 and TGF- β , reiterating the fact that IL-10 inhibition is more important than Th1 amplification in the management of VL [11]. Other liposomal formulations, such as CpG oligodeoxynucleotide [12] and liposomal palmitoyl tuftsin [13] when applied with miltefosine, could also induce an overall antileishmanial response through enhancement of Th1 cytokines, nitric oxide, reactive oxygen species and H₂O₂. Pam3Cys, an in-built immunoadjuvant and a Toll-like receptor 2 ligand, when combined with miltefosine could demonstrate an enhanced antileishmanial activity through Th1 immunomodulation and production of reactive oxygen/nitrogen species and H₂O₂ [14]. Two analogs of muramyl dipeptide viz. N.Ac-norMur-MeVal-D-isoGln (86/448) and N.AcMur-Acc-D-isoGln (89/729) could produce a typical Th1 response and a down-regulation of cytokines IL-4 and IL-10, when used as an immunomodulator with sodium

stibogluconate [15]. Immunotherapy also has its implications for post-kala-azar dermal leishmaniasis, a well-recognized complication following successful treatment of VL, where combination therapy of sodium stibogluconate with alum-precipitated autoclaved *Leishmania major* (Alum/ALM) plus BCG could induce a superior post-kala-azar dermal leishmaniasis healing compared with the drug alone through an IFN- γ -mediated Th1 immune response [6]. Apart from IFN- γ and IL-10, IL-2 also has therapeutic potential, as its neutralization led to the inhibition of IL-10 expansion and subsequent control of infection in experimental VL [16]. Other suppressive cytokines were also examined for their immunotherapeutic efficacy, but inhibition of IL-13 or TGF- β had no parasitocidal effect, and an anti-IL-4 mAb had no bearing on the disease outcome [6]. Although these observations support immunotherapy as a promising alternative to conventional chemotherapy against VL, problems, particularly with cytokine therapy, have to be addressed before its application. Since recombinant cytokines have an extremely short half-life, multiple doses have to be administered, which may cause infusion-related side effects. This aspect should be addressed properly before their clinical application. Alternately, T cells such as CD4, CD8 and NK cells, as well as dendritic cells (DCs), that are the sources of these regulatory cytokines could be used in immunotherapy. DCs, being the most potent APCs, play an important role in the activation of T, B and NK cells and these activated cells could mount a protective Th1 immune response against VL. Bone marrow-derived DCs pulsed with soluble *Leishmania donovani* antigen when injected in combination with antimonials reduced both hepatic and splenic parasite burden significantly [6]. Further studies confirmed that this therapeutic efficacy of bone marrow-derived DCs depends on IL-6 and in presence of this cytokine, it could also inhibit the expansion of IL-10-producing CD4⁺ T cells [17]. Thus the future of DC-based immunotherapy appears exciting and it can be looked upon as a prospective vaccine against VL. NK T cells also have therapeutic potential, as synergy of its β -(1–4)-galactose terminal with Toll-like receptor 4 and subsequent detection of glycosphingophospholipid antigen of *L. donovani* parasite could initiate intracellular signaling pathways necessary for parasite killing [18]. Application of recombinant human GM-CSF, along with antimonials, could also be an interesting alternative as it could significantly increase neutrophil, eosinophil and monocyte

counts leading to resolution of the disease in Brazilian VL patients [19]. Apart from these, chimeric fusion proteins have been used as a treatment option. The natural ligand for OX40, a member of the TNF receptor superfamily, OX40L, is expressed mainly on APCs. A chimeric fusion protein made of OX40L and the Fc region of immunoglobulin (OX40L–Fc), when administered in *L. donovani*-infected mice, could lead to CD4⁺ T-cell proliferation and killing of parasites. Furthermore, mAbs targeted against CTLA-4, an inhibitory receptor on T cells, could also enhance leishmanicidal activity. Both OX40L–Fc and anti-CTLA-4 treatments exhibited similar therapeutic efficiency. However, although a combination of OX40L–Fc with antimonials could not further enhance the parasiticidal efficacy, a combination of anti-CTLA-4 with antimonials had a mild additive effect [20].

Results from both murine and human studies suggest the emergence of immunotherapy, involving different agonists and antagonists to the cytokines and cells involved in the course

of disease progression, as a promising treatment option against VL. Administration of immunomodulators and novel compounds along with different first-line drugs could not only help in increasing treatment efficacy, but also in reducing total drug dose, as well as toxicity and adverse side effects involved with conventional chemotherapy. However, detailed trials in both animals and humans should be carried out to standardize the dose and route of administration of these agents and determine the feasibility of immunotherapy as a potent treatment for long-term cure.

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