Research brief

Leishmania donovani: Effect of therapy on expression of CD2 antigen and secretion of macrophage migration inhibition factor by T-cells in patients with visceral leishmaniasis

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Abstract

Visceral leishmaniasis (VL) commonly known as Kala-azar in India is one of the several clinically important infections, where Th1 sub-population of CD4+ T-cells, despite a pre-requisite, fails to express macrophage migration inhibition factor (MIF) and interferon-γ which both activate the macrophage and coordinate the immune response to intra-cellular Leishmania sp. Expression of CD2 receptors before and after antileishmanial therapy on CD4+ T-cells of VL patients and their corresponding effect on MIF were examined. Before treatment the number of T-cells expressing CD2 was low which incorporated insignificant MIF response. The immunological reconstitution was, however, observed after treatment as manifested through upregulation of CD2+ T-cells with pronounced MIF generation response. The study, therefore, identifies a possible role of CD2 antigen in immunity to VL.

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Keywords: Visceral leishmaniasis; CD2; Macrophage migration inhibition factor

The initial encounter of antigen specific T-cell receptors and CD4 co-receptors on the surface of Leishmania infected macrophages is important for activation of Th1 cells for clonal expansion due to IL2 synthesis in the presence of co-stimulation provided by macrophages (Rudd, 1996). The crucial step to initiate these events appears to be the transient binding of T-cells to the antigen-presenting cell. Cell surface adhesion molecules of the immunoglobulin super-family—lymphocyte function associated antigen-1 (LFA-1), CD2, and intracellular activation motif (ICAM-3)—can mediate T-cell adhesive interactions to enable an immune response to occur (Dustin and Springer, 1989; Hogg and Landis, 1993). Many reports suggested the role of CD2 in up-regulation of protein kinase C (PKC) mediated phosphorylation in CD4 cells, which results in increased IL2 production and its receptor expression (Cantrell et al., 1987; Droge, 1986). The present study was undertaken to explore the role of CD2 antigen co-receptors in T-cell non-responsiveness to L. donovani antigen in visceral leishmaniasis (VL) patients.

The experiment was designed to evaluate the level of the CD2+ T-cell sub-population and its effect on the percentage release of microphage migration inhibition factor (MIF) from T-cell of active VL patients and VL patients cured after “successful chemotherapy,” i.e., those with regressed spleen, disappearance of fever, and a negative parasitological status as determined by microscopical examination of splenic aspirate smears for Leishmania parasites. Thirty subjects of both sexes and from all age groups were studied, of which 10 represented a target...
were cultured in RPMI-1640 medium (Hi-media, India) supplemented with 2 mM L-glutamine (Hi-media, India), 10% fetal calf serum (Life Technologies, Gaithersburg, USA), 5 × 10^{-2} mM of 2-mercaptoethanol (Sigma) and 50 μg/ml gentamycin, 100 U/ml penicillin, 100 g/ml streptomycin, and then adjusted to pH 7.4 with 2 N HCl. Cells (5 × 10^6/ml^{-1}) pulsed with *L. donovani* promastigotes in responder (PBMC) to stimulator (*L. donovani* parasite) ratio of 100:1 for 2 h at 37 °C were subsequently cultured in 96-well round bottomed plates pre-coated with anti-CD2 (LFA-2) antibodies (4 μg/ml).

Control cultures were set up in medium alone or medium containing phytohaemagglutinin (PHA) 20 μg/ml for 48 h. All cultures were maintained at 37 °C in a water saturated air atmosphere containing 5% CO_2. MIF was measured in all subjects as an index of cell mediated immunity (Clausen, 1971). In brief, wells were cut in a preparation of agarose in a petri dish (15 × 90 mm) for a reaction of cultured lymphocyte cell suspension in the presence and absence of mouse antihuman CD2 antibody. After 24 h incubation at 5% CO_2 in air at 37 °C, further incubation of MIF plates was stopped and the T-cell reactivity was assessed by measuring MIF, a glycoprotein lymphokine in percentage value, after fixation and staining in 0.37% formaldehyde and gentian violet suspension. The diameter of the migration areas was measured by ocular meter and the percentage of migration inhibition was calculated as follows:

\[
\text{Migration inhibition} = \frac{1 - \text{mean migration diameter of cells in the presence of antigen}}{\text{mean migration diameter of cells in the absence of antigen}} \times 100.
\]

<table>
<thead>
<tr>
<th>Category</th>
<th>CD2^+ T-cells (μl)</th>
<th>MIF (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unstimulated</td>
<td>CD2 stimulated</td>
<td>PHA stimulated</td>
<td></td>
</tr>
<tr>
<td>Untreated VL (n=10)</td>
<td>654 ± 175.87</td>
<td>16.88 ± 4.56</td>
<td>37.62 ± 3.26</td>
<td>37.31 ± 6.89</td>
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<tr>
<td>Cured VL (n=10)</td>
<td>1211 ± 206</td>
<td>40.43 ± 15</td>
<td>44.43 ± 15.8</td>
<td>40.96 ± 14.81</td>
<td></td>
</tr>
<tr>
<td>Healthy control (n=10)</td>
<td>1877 ± 823.58</td>
<td>25.57 ± 3.36</td>
<td>33.63 ± 6.51</td>
<td>35.63 ± 6.80</td>
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</tr>
</tbody>
</table>

Table showing low level of CD2^+ T-cells during active VL infection associated with poor T-helper cell response for MIF release, which increased when MNC were stimulated with anti-CD2 antibodies.
of MIF by T-cells in the active stage of the disease (Table 1). After successful chemotherapy, the number of T-cell expressing CD2 antigen increased progressively by about twofold with corresponding increase in MIF secretion by these cells. It was also apparent that low number of cells expressed CD2 antigen, so they were unable to trigger activation events in T-lymphocyte for active cytokine response to L. donovani antigen. Previously, Ghosh et al. (1996) reported a reduction in CD2 sub-population of T-cells in Indian patients. As a result, T-cells were not stimulated and MIF response was very low. Earlier studies have also demonstrated that stimulation via CD2 facilitated signal transduction pathways (Gouvello et al., 1998; Monostori et al., 1990), which served to augment IL12 responsiveness of activated T-cells (Gollo et al., 1996) and reversed T-cell anergy (Boussiotis et al., 1994).

However, the number of T-cells positive for CD2 started increasing after treatment, so the CD2 antigens were able to stimulate T-cells. As a result, MIF level increased. The expression or disappearance of CD2 antigen on the T-cells in immune mediated disorders such as VL can therefore be determined to monitor the response of patients to therapeutic agents.

To further determine the role of CD2 antigen, we examined the production of MIF by exposing the T-cells of patients in vitro to agnostic anti-CD2 mAb. We preferred MIF on account of its simplicity, ease of use in laboratories with limited facilities and its relative sensitivity when compared to other cytokine based detection assays such as interferon-g (IFN-g) evaluation, which are generally less affordable in remote rural areas where modern methods cannot be used. It was observed that patients with active infection potentially generated more MIF, which was secreted about twofold less in absence of anti-CD2 exposure. It was also observed that cells of active cases responded as well to anti-CD2 antibody as did cells from cured cases.

CD2 antigen has shown to be important in initiating phosphorylation in proteins of CD4 molecules. In these studies, the effect of CD2 antigen is obtained through regulation of diacylglycerol (DAG), which seems to be the intracellular activator of PKC (Pantaleo et al., 1987). It is appreciated that stimulation of PKC through CD2 antigen would lead to production of Th1 cytokines, which are important for resistance to intracellular pathogens. However, stimulation through CD2 can also affect expression of CD28 which can further enhance T-cell response. In addition, the CD2 pathway can also lead to direct stimulation of T-cells and may have a role in the generation of effector cells. This study identifies a crucial role for the CD2 antigen in immune responses to Leishmania.

References


