HUMAN SCHISTOSOMIASIS MANSONI: STUDIES ON IN VITRO GRANULOMA MODULATION

JUÇARA C. PARRA; BARBARA DOUGHTY*; DANIEL G. COLLEY** & GIOVANNI GAZZINELLI

Centro de Pesquisas René Rachou – FIOCRUZ, Caixa Postal 1743, 30161-970 Belo Horizonte, MG, Brazil
*Dept. of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Texas A & M University, College Station, TX 77843, U.S.A. **VA Medical Center and Vanderbilt University School of Medicine, Nashville, TN 37212, U.S.A.

Infection with Schistosoma mansoni induces humoral and T cell mediated responses and leads to a delayed hypersensitivity that results in granulomatous inflammatory disease around the parasite eggs. Regulation of these responses resulting in a reduction in this anti-egg inflammatory disease is apparently determined by idiotypic repertoires of the patient, associated with genetic background and multiple external factors. We have previously reported on idiotype/anti-idiotype-receptor interactions in clinical human schistosomiasis. These findings support a hypothesis that anti-SEA cross-reactive idiotypes develop in some patients during the course of a chronic infection and participate in regulation of anti-SEA cellular immune responses. We report here on experiments which extend those observations to the regulation of granulomatous hypersensitivity measured by an in vitro granuloma model. T cells from chronic intestinal schistosomiasis patients were stimulated in vitro with anti-SEA idiotypes and assayed in an autologous in vitro granuloma assay for modulation of granuloma formation. These anti-SEA idiotype reactive T cells were capable of regulating autologous in vitro granuloma formation. Both CD4 and CD8 T cells could be activated to regulate granuloma formation. This regulatory activity, initiated with stimulatory anti-SEA idiotypic antibodies, was antigenically specific and was dependent on the presence of intact (F(ab')2 immunoglobulin molecules. The ability to elicit this regulatory activity appears to be dose dependent and is more easily demonstrated in chronically infected intestinal patients or SEA sensitized individuals. These data support the hypothesis that anti-SEA cross reactive idiotypes are important in regulating granulomatous hypersensitivity in chronic intestinal schistosomiasis patients and these cross-reactive idiotypes appear to play a major role in cell-cell interactions which result in the regulation of anti-SEA cellular immune responses.

Key words: Schistosoma mansoni – granuloma – regulation – idiotypic antibodies

The granulomatous inflammation that occurs around parasite eggs is considered the basic lesion in schistosomiasis pathology and is mediated by immune response to soluble egg antigens (SEA). As the disease progresses from an acute to a chronic phase, these anti-SEA responses are modulated by specific immunoregulatory events (Andrade & Warren, 1964). Multiple factor are involved in this regulation which is mediated primarily by cellular response (Boros, 1986; Colley, 1976; Colley, 1981a, b).

Except for the work of Rocklin et al. (1980), who observed a correlation between suppression of SEA-stimulated cultures and smaller rectal egg-induced granulomas, most information about granuloma formation/modulation in humans has been obtained by examining infected patients peripheral blood mononuclear cells (PBMC) reactivity to antigen-conjugated polycrylamide beads in a so-called in vitro granuloma assay (Doughty et al., 1989). The cellular reactivity was determined by morphological observations based on the following criteria: the number of cells binding to the beads; visual evidence of blast transforming
TABLE

Summary of participating cell populations

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Cell population</th>
<th>Granuloma index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Unfractionated</td>
<td>2.81 ± .16</td>
</tr>
<tr>
<td></td>
<td>Macrophage depleted</td>
<td>1.45 ± .09</td>
</tr>
<tr>
<td>2</td>
<td>Unfractionated</td>
<td>2.10 ± .11</td>
</tr>
<tr>
<td></td>
<td>Macrophage depleted</td>
<td>1.03 ± .05</td>
</tr>
<tr>
<td>3</td>
<td>Unfractionated</td>
<td>3.02 ± .07</td>
</tr>
<tr>
<td></td>
<td>B cell depleted (T+, Mø+)</td>
<td>3.18 ± .05</td>
</tr>
<tr>
<td></td>
<td>T cells (Ig-, Mø-)</td>
<td>1.05 ± .04</td>
</tr>
<tr>
<td></td>
<td>T cell + Mø</td>
<td>3.40 ± .05</td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td>3.00 ± .06°</td>
</tr>
</tbody>
</table>

a: macrophages (Mø) purified by adherence to plastic demonstrated enhanced binding to both SEA conjugated beads and unconjugated polyclonal antibody beads. This apparent non-specific binding elevated the granuloma index. However, the cellular reactivity observed was always five or more cells binding (never fewer nor more) to the beads (classification #3).

cells accompanied by cellular migration toward the beads; and adherent cell layers surrounding the beads. A numerical score was developed to clarify each cell-bead reaction observed (Doughty et al., 1989). Experimental manipulations of cells for the purpose of studying the regulation of granulomatous hypersensitivity were carried out in a completely autologous system.

Previously we have shown that the in vitro granuloma response is dependent on SEA-specific CD4+ T cells and macrophages. PBMC's depleted of B cells on an anti-human F(ab) '2 Ig column gave granuloma indices equivalent to an unfractionated PBMC population (Table) (Doughty et al., 1987). These studies suggested also that immunoregulation in human chronic schistosomiasis is predominantly cellular in nature, particularly implicating a CD8+ T lymphocyte as the ultimate effector of the regulation of this reactivity. Non-specific humoral factors can also modulate the immune response at the in vitro granuloma level. For example, when PBMC are treated with sera from infected patients they were able to cause the inhibition of in vitro granuloma formation (Goes et al., 1991). Similar results were observed upon treatment of PBMC with isolated immune complexes (IC), or with manufactured IC of SEA and purified IgG from pooled chronic schistosomiasis sera. Since this effect is reduced by addition of indomethacin to the granuloma culture it was suggested that circulating IC may regulate granulomatous hyper-
sensitivity to S. mansoni eggs by inducing macrophages to secrete suppressive prostaglandins (Goes et al., 1991).

ROLE OF ANTI-IDIOPTYPIC T CELLS IN GRANULOMA MODULATION

Idiopitic/anti-idiotype interactions have been reported in both experimental and human schistosomiasis as well as other chronic endemic parasitic infections such as Trypanosoma cruzi (Colley, 1990).

It has been reported that anti-idiotype (anti-Id) CD4+ and CD8+ T cell-subsets can down-regulate autologous granuloma formation in vitro when previously incubated with either a human IgG2 anti-SEA mAb or polyclonal anti-SEA antibodies, immunoaffinity-purified from pooled of sera from chronically infected intestinal patients. These antibodies suppressed the autologous granuloma formation by interaction with anti-Id T lymphocytes. This effect was not observed with Fab fragments of the anti-SEA antibodies, suggesting that cross-linking of T cell membrane components is required to induce this phenomenon, as it is for the induction of anti-id T cell proliferation (Parra et al., 1988). The Id/anti-Id interaction described is specific because it did not affect the PPD-bead granuloma system and did not occur with normal human IgG (Parra et al., 1991).

In summary, these studies strongly support the participation of Id/anti-Id interactions at the T cell level in these immuno-regulatory mechanisms. The absence of such mechanisms in S. mansoni infection has been correlated with severe hepatosplenic disease (Colley et al., 1976; Montesano et al., 1989). This interpretation is supported by our preliminary data which demonstrate a failure of anti-SEA idiotypic preparations to induce suppression of in vitro granuloma formation with PBMC from patients with hepatosplenic schistosomiasis.

REFERENCES


