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HUMAN SCHISTOSOMIASIS MANSONI: STUDIES ON *IN VITRO* GRANULOMA MODULATION

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Infection with Schistosoma mansoni induces humoral and T cell mediated responses and leads to a delayed hipersensitivity that results in granulomatous inflamatory disease around the parasite eggs. Regulation of these responses resulting in a reduction in this anti-egg inflamatory 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 repport 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 hypersensitivy 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 polyacrylamide 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

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TABLE

Summary of participating cell populations

Patient #	Cell population	Granuloma index
1	Unfractionated	2.81 ± .16
	Macrophage depleted	1.45 ± .09
2	Unfractionated	2.10 ± .11
	Macrophage depleted	1.03 ± .05
3	Unfractionated	3.02 ± .07
	B cell depleted (T+, Mø+)	3.18 ± .05
	T cells (Ig–, Mø–)	1.05 ± .04
	T cell + Mø	3.40 ± .05
	Macrophages	3.00 ± .0[a]

a: macrophages (Mø) purified by adherence to plastic demonstrated enhanced binding to both SEA conjugated beads and unconjugated polyacrylamide 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 sorrounding 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 pacients 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 hypersensitivity to *S. mansoni* eggs by inducing macrophages to secrete suppressive prostaglandins (Goes et al., 1991).

ROLE OF ANTI-IDIOTYPIC T CELLS IN GRANULOMA MODULATION

Idiotypic/anti-idiotypic 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-idiotypic (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 correlatd 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

ANDRADE, Z. & WARREN, K. S., 1964. Mild prolonged schistosomiasis in mice: alterations in host response with time and the development of portal fibrosis. *Trans. R. Soc. Trop. Med. Hyg., 58:* 53-57.

BOROS, D. L., 1986. Immunoregulation of granuloma formation in murine schistosomiasis mansoni. *Ann. NY Acad. Sci., 465:* 313-323.

COLLEY, D. G., 1976. Adoptive suppression of granuloma formation. *J. Exp. Med., 143:* 696-700.

COLLEY, D. G., 1981a. Immune responses and immunoregulation in experimental and clinical schistosomiasis, p. 1-81. In J. M. Mansfield, *Parasitic Diseases, vol. 1. The Immunology*. Marcel Dekker, Inc., New York.

COLLEY, D. G., 1981b. T lymphocytes which contribute to the immuno-regulation of granuloma formation in chronic murine schistosomiasis. *J. Immunol., 126:* 1465-1468.

COLLEY, D. G., 1990. Occurrence, roles, and uses of idiotypes and anti-idiotypes in parasitic diseases, p. 71-1055. J. Cerny & J. Hiernaux (eds). *Idiotypic Network and Diseases*. American Society for Microbiology, Washington, DC.

COLLEY, D. G. & GAZZINELLI, G., 1987. Granulomatous hypersensitivity to *Schistosoma mansoni* egg antigens in human schistosomiasis. I – Granuloma formation and modulation around polyacrylamide antigen-conjugated beads. *Mem. Inst. Oswaldo Cruz, 82* (Suppl. IV): 47-54.

DOUGHTY, B. L.; GOES, A. M.; PARRA, J. C.; ROCHA, R. S.; CONE, C.; COLLEY, D. G. & GAZZINELLI, G., 1989. Anti-idiotypic T cells in human schistosomiasis. *Immunol. Inv., 18:* 373-388.

GOES, A. M.; GAZZINELLI, G.; ROCHA, R.; KATZ, N. & DOUGHTY, B. L., 1991. Granulomatous hypersensitivity to *Schistosoma mansoni* egg antigens in human schistosomiasis. III – *In vitro* granuloma modulation induced by immune complexes. *Am. J. Trop. Med. Hyg., 44:* 434-443.

MONTESANO, M. A.; LIMA, M. S.; CORREA-OLIVEIRA, R.; GAZZINELLI, G. & COLLEY, D. G., 1989. Immune responses during human schistosomiasis mansoni. XVI – Idiotypic differences in antibody preparations from patients with different clinical forms of infection. *J. Immunol., 172:* 2501-2506.

PARRA, J. C.; GAZZINELLI, G.; GOES, A. M.; MOYES, R. B.; ROCHA, R.; COLLEY, D. G. & DOUGHTY, B. L., 1991. Granulomatous hypersensitivity to *Schistosoma mansoni* egg antigens in human schistosomiasis. II – *In vitro* granuloma modulation induced by polyclonal idiotypic antibodies. *Immunol.,* December.

PARRA, J. C.; LIMA, M. S.; GAZZINELLI, G. & COLLEY, D. G., 1988. XV – Anti-idiotypic T cells can recognize and respond to anti-SEA idiotypes directly. *J. Immunol., 140:* 2401-2405.

ROCKLIN, P. E.; BROWN, A. P.; WARREN, K. S.; PELLEY, R. P.; HOUBA, V.; SIONGOK, T. K. A.; OUMA, J.; STURROCK, R. F. & BUTTERWORTH, A. E., 1980. Factors that modify the cellular-immune response in patients infected by *Schistosoma mansoni. J. Immunol., 125:* 1916-1923.