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GRANULOMATOUS HYPERSENSITIVITY TO *SCHISTOSOMA MANSONI* EGG ANTIGENS IN HUMAN SCHISTOSOMIASIS. I. GRANULOMA FORMATION AND MODULATION AROUND POLYACRYLAMIDE ANTIGEN-CONJUGATED BEADS

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We have developed an in vitro *model of granuloma formation for the purpose of studying the immunological components of delayed type hypersensitivity granuloma formation in patients infected with* Schistosoma mansoni. *Our data show that 1) granulomatous hypersensitivity can be studied by examining the cellular reactivity manifested as multiple cell layers surrounding the antigen conjugated beads; 2) this reactivity is a CD 4 cell dependent, macrophage dependent, B cell independent response and 3) the* in vitro *granuloma response is antigenically specific for parasite egg antigens.*

*Studies designed to investigate the immune regulation of granulomatous hypersensitivity using purified populations of either CD4 or CD8 T cells have demonstrated the complexity of cellular interactions in the suppression of granulomatous hypersensitivity. The anti-*S. mansoni *egg immune responses of individual patients with chronic intestinal schistosomiasis can be classified either as soluble egg antigen (SEA) hypersensitive with maximal granulomatous hypersensitivity or SEA suppressive with activation of the T cell suppressor pathway with effective SEA granuloma modulation. Our data suggest that T cell network interactions are active in the generation of effective granuloma modulation in chronic intestinal schistosomiasis patients.*

The human immune response to infection with *Schistosoma mansoni* is complex due to the intensity of infection, age of the individual at the time of first exposure, previous exposure *in utero* to either antigen or maternal anti-schistosome antibodies, multiple exposures, genetic composition of the individual, and length of infection (Colley, 1981; Nash et al., 1982; Colley, 1987; Capron, 1987). Many investigators have reported on patients' immune responsiveness to crude and fractionated schistosome antigens (Reviewed by Colley, 1987 and Capron, 1987). Specific immunological mechanisms have been described for protective immunity as well as immunopathological mechanisms relating to morbidity (Capron et al., 1977; Abel-Salam et al., 1979, 1986; Dunn et al., 1979; Ellner et al., 1980; Galvão-Castro et al., 1981; Kamal & Higashi, 1982; Tweardy et al., 1983; Butterworth et al., 1985; Colley et al., 1986; Garcia et al., 1986). The anti-egg host inflammatory response has been shown to be highly regulated by suppressive mechanisms in long term chronic infections (Phillips & Lammie, 1986). It would appear from studies examining cell-mediated responses, that patients with hepatosplenic disease (ambulatory, not hospitalized) have unregulated responses to SEA. These studies suggest that morbidity may be associated with a lack of effective regulation of granulomatous hypersensitivity (Colley et al., 1986). It was, therefore, of interest to us to pursue a model that would enable us to examine the cellular basis of granulomatous hypersensitivity and the cellular mechanisms of the immunoregulation of that response in human schistosomiasis.

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MATERIALS AND METHODS

Study population: patients were selected for active schistosomiasis mansoni infections based on parasitological stool examination for the presence of *S. mansoni* eggs by the Kato-Katz technique (Katz et al., 1972). Ages of chroni-

cally infected schistosomiasis patients ranged from 18-43 years and quantitative egg counts were from 12-516 eggs/gram. Uninfected, normal controls were individuals selected either from the laboratory in Belo Horizonte, Minas Gerais or from the laboratory in College Station, Texas. Control individuals had no history of exposure to *S. mansoni* and also showed no antiparasite antibody responses or lymphocyte responsiveness to soluble egg antigens (SEA). Informed consent was obtained from each subject before inclusion into the study, and active schistosomiasis patients were given Oxamniquine therapy after diagnosis.

Antigens: soluble egg antigens were isolated from crude homogenates of *S. mansoni* eggs according to published procedures (Doughty & Phillips, 1982). Purified protein derivative from *Mycobacterium tuberculosis* was purchased from Connaught Laboratories, Canada.

Conjugation of SEA onto polyacrylamide beads: polyacrylamide beads, 40-60 microns in diameter, were obtained from RioRad, Bio-Gel P-4 (#150-0459), Richmond California, 94804. The beads were then sterilized and washed in 0.5 M bicarbonate buffer by gentle centrifugation. 200 mgs of beads were incubated for 4 hours in a 63°C water bath with gentle agitation and then washed three times with sterile distilled water and then mixed with 20 mg of antigen (SEA or PPD) and 100 mg of EDAC (1-ethyl 3-(3)dimethylaminopropyl) carbodiimide HCL in 100 mls of slightly acidic (pH 6.5-6.8) sterile distilled water. The mixture was gently rotated for 18 hours at 4°C. The beads were then washed in sterile phosphate buffered saline to remove reaction by products and stored at 4°C in sterile phosphate buffered saline with 0.1% sodium azide until needed.

Cell preparations: peripheral blood mononuclear cells (PBMN's) were isolated from heparinized blood by Ficoll-diatrizoate density gradient centrifugation (LSM: Organon-Teknika, Charleston, SC) at a 2:1 v:v ratio (Gazzinelli et al., 1983). Adherent monocytes were removed from PBMN's by two cycles of adherence to plastic. Purity of cell separations were determined by indirect immunofluorescent staining of monocytes with an antimonocyte specific monoclonal antibody (B52.1.1). Ig+ cells were removed on an anti-human Ig F(ab)'2 column. T cell populations differentiated by the cell surface markers CD4 and CD8 were purified on Degalon bead columns using monoclonal antibodies OKT4, B66.6.1, OKT8, B116.1.1 (Duarte et al., 1981). OKT4 and OKT8 were purchased from American Type Culture Collection (CRL #8002 and CRL #8014) and were produced from mouse ascitic fluid. B66.6.1 and B116.1.1 were monoclonal antibodies produced against CD4 helper cells and CD8 suppressor cells. These antibodies (B66.6.1, B116.6.6 and B52.1.1) were a generous gift from Dr Giorgio Trinchieri, Wistar Institute, Philadelphia, PA.

In vitro *SEA polyacrylamide bead cultures:* two hundred SEA polyacrylamide beads were added in a volume of 100 μl into the bottom of 24 well tissue culture plates (Costar #3524). PBMN's from either schistosomiasis cell donors or uninfected controls were added into the culture well in a final volume of 2 ml. Culture medium contained 10% heat inactivated AB+ human serum, 1.6% L-glutamine (prepared from a stock of 200 mM), 3% antibiotic-antimycotic (prepared from a stock containing 10,000 U penicillin, 10 mg streptomycin, 25 μg Fungizone/ml) in RPMI 1640. Every experimental and control group was set up in triplicate and maintained at 37°C in a CO_2 incubator in an atmosphere of 5% CO_2 in air. *In vitro* granuloma reactivity was evaluated on day 5, 10, or 15 after culture initiation by quantitation of cellular reactivity around the beads after viewing the cultures in a phase contrast inverted tissue culture microscope (Leitz Diavert or Olympus IMT-2). Cellular reactivity was determined by morphological observation based on the following criteria: the number of cells binding to the beads, visual evidence of blast transforming cells accompanied by cellular migration, and adherent cell layers surrounding the beads. 300 separate determinations of cellular reactivity were made for each experimental group. A numerical score equivalent to the following classification was assigned to each cell – bead reaction observed.

Classification of *in vitro* bead granulomas:

1. No cells binding to the bead.
2. <5 cells binding to the bead.
3. >5 cells binding to the bead.
4. >5 cells binding to the bead accompanied by a circumoval mononuclear cell migration and blast transformation.
5. Adherent cell layer attached to the bead accompanied by circumoval mononuclear cell migration.

6. Multiple cell layers surrounding the bead accompanied by mononuclear cell migration.

The total score is then summed and the resultant mean expressed as a Granuloma Index (G.I.). All SEA bead culture reactivity was compared to the reactivity of beads with no antigen as an internal control on SEA immune reactivity versus non-specific binding of activated macrophages. The statistical significance was determined between groups by the Student's T test.

Autologous granuloma suppression: experimental manipulations of cells for the purpose of studying the regulation of granulomatous hypersensitivity were carried out in a completely autologous system. Unfractionated PBMN's, CD4 cell lines or CD8 cell lines were stimulated with SEA or pooled human anti-SEA monoclonal antibodies and then tested in a co-culture assay with freshly isolated autologous unfractionated PBMN's, or freshly isolated and purified CD4 cells and monocytes. Determination of autologous granuloma suppression was carried out in experiments using a fixed number of unstimulated cells co-cultured with either various numbers of SEA stimulated or anti-SEA stimulated cell lines. The percentage suppression was determined by calculating the experimental granuloma index, subtracting the granuloma index of the mixed population from the unfractionated granuloma index, dividing that number by the experimental granuloma index and multiplying by 100.

EBV induced lymphoblastoid cell transformation: the establishment of B cell lymphoblastoid cell lines was achieved by culturing 5 x 10^6 B cells in a 1 1/2 ml volume of medium containing RPMI 1640, 10% FCS and 0.5 mls of B95-8 supernatant containing Epstein Barr virus (EBV) for two hours. After incubation with EBV, B cells were recovered and dispensed into 96 well microtiter plates at a density of 2 x 10^4 cells/well. The transformation of B cells to lymphoblastoid cells was determined by visual observation of cell aggregation, pH change of sodium bicarbonate buffered medium and increased cell numbers. Once cell lines covered the bottom of the well, they were expanded into 24 well plates (Costar #3524) and subcultured by limiting dilution cloning.

Immortalization of anti-SEA EBV transformed cell lines: supernatants from EBV transformed cell lines were tested for anti-SEA antibody reactivity in an ELISA assay (Gazzinelli et al., 1985). Positive anti-SEA antibody producing cell lines were expanded and then fused with SHM-D33, a mouse-human hetero-myeloma (American Type Culture Collection # CRL-1668), according to the procedures of Teng et al., 1983. Fused cells were then plated at a cell density of 3.0 x 10^5 cells/well into a 96 flat bottomed microtiter plate. Cells were supplemented with fresh HAT medium twice weekly for the first two weeks and then once weekly thereafter. Growth characteristics, chromosome stability and quantitative anti-body production were important considerations in selecting hybrids to clone and characterize (Hoffman & Hirst, 1985; Kozbor et al., 1984; Edwards et al., 1982).

RESULTS

Granuloma indices of Schistosoma mansoni *infected patients:* the granuloma index was calculated from SEA conjugated bead cultures on day 5 after culture initiation at a total cell concentration of 1 x 10^6 PBMN's/well. The age of the patients ranged from 16-43 years, and the granuloma indices ranged from 2.02-4.37. Seven of the twenty-five patients had quantitative egg counts of $>$ 100 eggs/g. There was no significant difference between the granuloma indices of the group of patients with egg counts of $<$ 100 eggs/g when compared to the seven patients with egg counts $>$ 100 (Table I).

TABLE I

Patient population	Mean granuloma index +/– SD
Normal uninfected	2.2 +/– .39 N = 8
Active chronic	3.1 +/– .56 N = 25

The statistical significance of the difference in the mean Granuloma Indices of the normal versus the infected patient population was calculated using the Student's T test and gave a significant p value of $<$.001.

Antigenic specificity and contributing cell populations in granuloma formation: the antigenic specificity of the cellular reactions was demonstrated by examining the *in vitro* granulomatous response of a *Schistosoma mansoni* infected patient with both SEA and PPD conjugated beads, and the *in vitro* granulomatous response of an uninfected individual that had a positive PPD skin test reactivity with both SEA and PPD conjugated beads. Both individuals showed strong cellular reactivity to the original immunizing antigen (Table II).

TABLE II

Antigenic specificity of *in vitro* granuloma formation

Cell donor	Bead/antigen	Classification	Granuloma index
S. mansoni Infected PPD skin test Negative	PB-SEA	Chronic intestinal	4.37 +/– .3
	PB-PPD		2.77 +/– .02
Uninfected PPD skin test Positive	PB-SEA	–	2.77 +/– .14*
	PB-PPD	–	4.27 +/– .3**

* Statistically significant difference at a p value $<.001$ between *in vitro* reactivity against SEA polyacrylamide beads in cultures from a chronically infected schistosomiasis patient and an uninfected, PPD skin test positive individual.

** Statistically significant difference at a p value $<.001$ between *in vitro* reactivity against PPD polyacrylamide beads in cultures from a chronically infected schistosomiasis patient and a PPD skin test positive individual.

Negative selection of macrophages from PBMN's reduced the ability of the remaining lymphocytes to form an *in vitro* granuloma. T cell depletions had the same effect (data not shown). B cell depletions, however, showed no reduction in granulomatous hypersensitivity. Highly purified T cells (> 95% CD3+) gave an equivalent *in vitro* granuloma reaction when mixed with purified macrophages. When macrophages were purified by adherence to plastic, recovered by gentle mechanical scrapping with a rubber cell scraper and co-cultured with either SEA conjugated beads or beads with no antigen, they showed an enhanced binding capacility (G.I. 3.0). We interpret this as possible non-specific activation as a result of experimental manipulations in the cell purification process. These cells did not exhibit any reactivity above the classification #3, but did show cells binding to each bead, which elevated the granuloma index but did not give the typical reactivity pattern of unfractionated PBMN's, or purified T cells and macrophages (Table III).

Autologous granuloma modulation using purified T cell populations: in order to test the suppressive capabilities of positively selected T cell populations, we produced antigen reactive CD4 cell lines and CD8 cell lines that were expanded on IL-2 for a period of two weeks. At this time, the patients returned to the clinic at the Centro de Pesquisas "Rene Rachou" in Belo Horizonte, Brazil in order to donate blood a second time. The T cell lines were then co-cultured with fresh unfractionated autologous PBMN's. Our results demonstrate that either purified CD4 or CD8 cells alone will not produce the typical cell reactivity observed in the unfractionated PBMN cell-SEA conjugated bead cultures (Table IV). A purified antigen-reactive T cell line will produce *in vitro* granulomas when co-cultured with purified monocytes. Certain patients also exhibit an ability to induce suppression when CD4 T cells are co-cultured with their own autologous PBMN's. Another group of patients demonstrated suppressor effector CD8 cell activity in autologous granuloma cultures.

TABLE III

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^+, $M\phi^+$)	3.18 +/– .05
	T cells (Ig^-, $M\phi^-$)	1.05 +/– .04
	T cells + $M\phi$	3.4 +/– .05
	Macrophages	3.0 +/– .0*

* Macrophages 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 manifested as five or more cells binding to the beads (classification #3).

TABLE IV

Analysis of effector cell functions with purified T cells isolated from PBMN's of patients infected with *Schistosoma mansoni*

Cell population and cell numbers	Monoclonal antibody used for selection	Functional assay T_{SI}	T_{DTH}	T_{SE}
Unfractionated PBMN's 1×10^6	none	–	+	–
CD4 cells	anti-CD4	–	–	–
CD8 cells	anti-CD8	–	–	–
Monocytes + CD4 cells	anti-CD4 + anti-Mϕ	–	+	–
Monocytes + CD8 cells	anti-CD8 + anti-Mϕ	–	–	–
CD4 inducer cell assayed in a dose response fashion with 1×10^6 unfractionated autologous PBMN's	anti-CD4	+	–	–
CD8 inducer cell assayed in a co-culture experiment with 1×10^6 unfractionated autologous PBMN's	anti-CD8	–	–	+

TABLE V

Analysis of effector cell functions with purified T cells isolated from PBMN's of patients infected with *Schistosoma mansoni*

Cell population and cell numbers	Monoclonal antibody used for selection	Functional assay T_{SI}	T_{DTH}	T_{SE}
anti-ID CD4 cells assayed with 1×10^6 autologous unfractionated PBMN's	anti-CD4	+	–	–
anti-ID CD4 cells assayed with 1×10^6 CD4 T cells and Mϕ	anti-CD4 anti-MO anti-SEA	–	+	–
anti-ID CD8 cells assayed with 1×10^6 autologous unfractionated PBMN's	anti-CD8 anti-SEA mAb	–	–	+
anti-ID CD8 cells assayed with purified CD4 cells and Mϕ	anti-CD8 anti-CD4 anti-Mϕ anti-SEA mAb	–	–	+
anti-ID CD4 cells assayed with CD4 sca specific T cells, Mϕ, and unstimulated CD8 cells	anti-CD8 anti-CD4 anti-Mϕ anti-SEA mAb	+	–	+

Role of anti-idiotypic T cells in autologous granuloma modulation: in order to analyze whether anti-idiotypic T cell subsets played a role in the suppressor cell modulation of granuloma formation, we produced and characterized anti-SEA human monoclonal antibodies. We selected five cloned hybrid cell lines and purified the monoclonal antibodies from culture supernatants. These five anti-SEA monoclonal antibodies were used to stimulate either CD4 or CD8 T cell lines and then tested in the autologous granuloma assay for either suppressor cell inducer activity or suppressor effector cell activity. Table V summarizes our results. Anti-idiotypic CD4 T cells were capable of inducing suppression with autologous PBMN's but failed to induce suppression when co-cultured with purified macrophages and freshly isolated CD4 T cells, indicating that activation of another T cell subset is necessary for effective suppression. Anti-idiotypic CD8 T cells were also suppressive when co-cultured with autologous unfractionated PBMN's and when assayed with freshly isolated autologous CD4 T cells and macrophages.

DISCUSSION

The *in vitro* model of granuloma formation which we have described in this report involves a circumoval cellular reaction around SEA conjugated polyacrylamide beads. This model reflects the delayed type hypersensitivity cellular components of granulomatous hypersensitivity. These components involve antigen processing, antigenic specificity, antigen sensitized T cells, and macrophages. Other cellular components such as eosinophils, fibroblasts, epitheloid cells and giant cell formation have been observed but are not reported here. Our studies support the experimental observations of other investigators who described the delayed type hypersensitivity components in experimental murine models of *Schistosoma mansoni* infections. We have shown that the *in vitro* granuloma response is dependent on SEA sensitized CD4 T cells and macrophages. PBMN's depleted of B cells on an anti-human F(ab)'2 Ig column gave granuloma indices equivalent to an unfractionated PBMN cell population. These results support the idea that delayed type granulomatous hypersensitivity does not require the presence of B cells, however, this does not rule out an important role for antibody in modulatory mechanisms.

Our patient population exhibited a large range of *in vitro* reactivity. The granuloma indices ranged from 2.02-4.37. There appeared to be a negative correlation (-0.379 with a $p < 0.05$) with patients having higher numbers of egg/g showing lower granuloma indices (data not shown). These observations are in contrast to previously reported work (Doughty et al., 1984). Those studies involved observations made on Egyptian school children, ages (6-13 years), with no observed significant differences between numbers of eggs/g and intensity of *in vitro* granuloma formation. The discrepancy in these observations could be the age of the patients, since a similar age group of Brazilian children showed no correlations in numbers of eggs/g and *in vitro* granuloma intensity (data not shown).

Admixture experiments using either purified CD4 or CD8 T cell lines expanded on SEA and/or IL-2 and autologous unfractionated PBMN's provided us with a method for analyzing the suppressor capabilities of certain cell populations. Our data are summarized in Table IV and V. CD4 T cells that were cultured with SEA and expanded on IL-2 after antigen stimulation showed suppressor inducer capabilities in some patients, but not all patients (two out of six), when co-cultured with autologous PBMN's and SEA conjugated beads. Three patients demonstrated strong CD8 suppressor effector cell activity when purified CD8 T cells were co-cultured with autologous PBMN cells and SEA conjugated beads. Our data indicate that chronically infected schistosomiasis patients have effective suppressor cell pathway activation and in most cases (one out of six patients showed no demonstrable suppressor cell activity) the suppressor cell activation, can be demonstrated to be predominantly at the inductive stage or the suppressor effector stage.

We were also interested in the role of anti-idiotypic T cells in autologous granuloma modulation. Previous investigators had reported that T lymphocytes from schistosomiasis mansoni patients could respond in a lymphocyte transformation assay to a polyclonal anti-SEA antibody preparation (Lima et al., 1986, Parra et al., 1988 and Gazzinelli et al., 1988). We, therefore, produced human anti-SEA monoclonal antibodies using anti-SEA EBV transformed human B cell lines as fusion partners for a mouse – human heteromyeloma SHM-D33 (Teng et al., 1983). Five anti-SEA

hybrid clones were chosen for high reactivity to SEA and good *in vitro* antibody production (5 μg/ml produced in culture supernatant/day). These antibodies were affinity purified, pooled in equal amounts and used to stimulate either purified CD4 or CD8 T cells from *S. mansoni* infected patients. These cell lines were expanded on pooled human monoclonal antibodies (150 μg/ml), and irradiated monocytes as idiotypic presenting cells. Purified anti-idiotypic CD4 cells were then co-cultured with autologous PBMN's or freshly isolated CD4 T cells and macrophages. Our data show that anti-idiotypic CD4 T cells could be stimulated to become suppressor inducer cells. The target cell of that suppressor induction is a CD8 T cell. We were also able to demonstrate an anti-idiotypic CD8 suppressor effector cell that acted directly on SEA specific CD4 T cells to suppress *in vitro* granuloma formation.

In conclusion, our data indicate that our *in vitro* model of granuloma formation is antigenically specific, requires SEA sensitized T cells and macrophages for effective *in vitro* cellular reactivity. Chronically infected schistosomiasis mansoni patients of the intestinal form, show a wide range of granuloma indices and also demonstrate suppressor cell pathway activation. These data indicate that our model of *in vitro* granuloma formation is a valuable assay for examining granulomatous hypersensitivity and immunoregulatory mechanisms associated with granuloma modulation.

REFERENCES

ABDEL-SALAM, E.; ISHAAC, S. & MAHMOUD, A. A. F., 1979. Histocompatibility-linked susceptibility for hepatosplenomegaly in human schistosomiasis. *J. Immunol., 123:* 1829-1831.

ABDEL-SALEM, E.; ABDEL KHALIK, A.; ABDEL-MEQUID, A.; BARAKAT, W. & MAHMOUD, A. A., 1986. Association of HLA Class I antigens (A1, B5, B8 and CW2) with disease manifestations and infection in human schistosomiasis mansoni in Egypt. *Tissue Antigens, 27:* 142-146.

BUTTERWORTH, A. E.; CAPRON, M.; CORDINGLY, J. S.; DALTON, P. R.; DUNNE, D. W.; KARIUKI, H. C.; KIMANI, G.; KOESCH, D.; MUGAMBI, M.; OUMA, J. H.; PRENTICE, M. A.; RICHARDSON, B. A.; ARAP SIONGOK, T. K.; STURROCK, R. F. & TAYLOR, D. W., 1985. Immunity after treatment of human schistosomiasis mansoni. II. Identification of resistant individuals, and analysis of their immune responses. *Trans. R. Soc. Trop. Med. Hyg., 79:* 393-408.

CAPRON, M.; CAMUS, D.; CARLIER, Y.; FIGUEIREDO, J. F. M. & CAPRON, M., 1977. Immunological studies in human schistosomiasis. II. Antibodies cytotoxic for *Schistosoma mansoni* schistosomules. *Am. J. Trop. Med. Hyg., 26:* 248-253.

CAPRON, A. & DESSAINT, P., 1987. Immunology of Schistosomiasis, p. 1-33. In E.J.L. Soulsby, *Immune Responses in Parasitic Infections: Immunology, Immunopathology and immunoprophylaxis, Vol. II Trematodes and Cestodes.* CRC Press, Florida.

COLLEY, D. G., 1981. Immune responses and immunoregulation in experimental and clinical schistosomiasis, p. 1-83. In J. M. Mansfield, *Parasitic Diseases* vol 1. Marcel Dekker, New York.

COLLEY, D. G.; GARCIA, A. A.; LAMBERTUCCI, J. R.; PARRA, J. C.; KATZ, N.; ROCHA, R. S. & GAZZINELLI, G., 1986. Immune responses during human schistosomiasis XII. Differential responsiveness in patients with hepatosplenic disease. *Am. J. Trop. Med. Hyg., 35:* 793-802.

COLLEY, D. G., 1987. Dynamics of the human immune response to schistosomes, p. 101-123. In R. A. Thompson *Recent Advances in Clinical Immunology,* vol 1. Churchill Livingstone, London.

DOUGHTY, B. L. & PHILLIPS, S. M., 1982. Delayed hypersensitivity granuloma formation around *Schistosoma mansoni* eggs *in vitro.* I. Definition of the Model. *J. Immunol., 128:* 30-36.

DOUGHTY, B. L.; ZODDA, Z. M.; EL KHOLY, A. & PHILLIPS, S. M., 1984. Delayed type hypersensitivity granuloma formation around *Schistosoma mansoni* eggs *in vitro* IV. Granuloma formation in human schistosomiasis. *Am. J. Trop. Med. Hyg., 33:* 1173-1177.

DUARTE, A. J.; CARPENTER, C. B. & STROM, T. B., 1981. Expression of T-cell differentiation antigens and Ia on rat cytotoxic T-lymphocytes. *J. Immunol., 128:* 580-584.

DUNN, M. A.; KAMEL, R.; KAMEL, I. A.; BIEMPICA, L.; KHOLY, A. E.; HAIT, P. K.; ROJKIND, M.; WARREN, K. S. & MAHMOUD, A. A., 1979. Liver collagen synthesis in schistosomiasis mansoni. *Gastroenterology, 76:* 978-982.

EDWARDS, P. A. W.; SMITH, C. M.; NEVILLE, A. M. & O'HARE, M. J., 1982. A human-human hybridoma system based on a fast growing mutant of the AKR-77 plasma cell leukemia-derived line. *Eur. J. Immun., 12:* 641-648.

ELLNER, J. J.; OLDS, G. R.; OSMAN, G. S.; EL KOHLY, A. & MAHMOUD, A. A. F., 1981. Dichotomies in the reactivity to worm antigen in human schistosomiasis. *J. Immunol., 126:* 309-312.

GALVÃO-CASTRO, B.; BINA, J. C.; PRATA, A. & LAMBERT, P. H., 1981. Correlation of circulating immune complexes and complement breakdown products with the severity of the disease in human schistosomiasis. *Am. J. Trop. Med. Hyg., 30:* 1238-1246.

GARCIA, A. A.; DA SILVA, A. L.; DE OLIVEIRA, L. R.; KATZ, N.; GAZZINELLI, G. & COLLEY, D. G., 1986. Immune responses during human schistosomiasis mansoni XVIII. Immunological status of spleen cells from hospital patients with hepatosplenic disease. *Scand. J. Immunol., 24:* 413-420.

GAZZINELLI, G.; KATZ, N.; ROCHA, R. S. & COLLEY, D. G., 1983. Immune responses during schistosomiasis mansoni. X. Production and standardization of an antigen-induced mitogenic

activity by peripheral blood mononuclear cells from treated, but not active cases of schistosomiasis. *J. Immunol., 130:* 2891-2895.

GAZZINELLI, G.; LAMBERTUCCI, J. R.; KATZ, N.; ROCHA, R. S.; LIMA, M. S. & COLLEY, D. G., 1985. Immune responses during human schistosomiasis mansoni. XI. Immunologic status of patients with acute infections and after treatment. *J. Immunol., 135:* 2121-2127.

GAZZINELLI, R. T.; PARRA, J. F. C.; CORREA-OLIVEIRA, R.; CANÇADO, J. R.; ROCHA, R.S.; GAZZINELLI, G. & COLLEY, D. G., 1988. Idiotypic/anti-idiotypic interactions in Schistosomiasis and/or Chagas disease. *Am. J. Trop. Med. Hyg.*, in press.

HOFFMAN, M. & HIRST, J. A., 1985. Principles of *in vitro* immunization of human B lymphocytes, p. 277-289. In E. Engleman, S. Foung, J. Larrick, and A. Raubitschek, *Human Hybrids and Monoclonal Antibodies*, Plenum Press, New York.

KAMAL, K. A. & HIGASHI, G. I., 1982. Suppression of mitogen-induced lymphocyte transformation by plasma from patients with hepatosplenic *Schistosomiasis mansoni:* role of immune complexes. *Parasite Immunol., 4:* 283-298.

KOZBOR, D.; TRIPPULI, P.; RODER, J. C. & CROCE, C. M., 1984. A human hybrid myeloma for production of human monoclonal antibodies. *J. Immunol., 133:* 3001-3005.

LIMA, M. S.; GAZZINELLI, G. & NASCIMENTO, E., 1986. Immune responses during human schistosomiasis mansoni. Evidence for antiidiotypic T lymphocyte responsiveness. *J. Clin. Invest., 78:* 983-988.

NASH, T. E.; CHEEVER, A. W.; OTTESEN, E. A. & COOK, J. A., 1982. Schistosome infection in humans: perspectives and recent findings. *Ann. Intern. Med., 97:* 740-754.

PARRA, J. C.; LIMA, M. S.; GAZZINELLI, G. & COLLEY, D. G., 1988. Immune responses during human schistosomiasis mansoni: XV. Anti-idiotypic T cells can recognize and respond to anti-SEA idiotypes directly. *J. Immunol.*, in press.

PHILLIPS, S. M. & LAMMIE, P. J., 1986. Immunopathology of granuloma formation and fibrosis in schistosomiasis. *Parasitology Today, 2:* 296-301.

TENG, N. N. H.; LAM, K. S.; RIERA, F. C. & KAPLAN, H., 1983. Construction and testing of mouse-human heteromyelomas for human monoclonal antibody production. *Proc. Natl. Acad. Sci. USA, 80:* 7308-7312.