INFECTION AND IMMUNITY, Feb. 1982, p. 465-470
0019-9567/82/020465-06$02.00/0

Vol. 35, No. 2

Alteration of Granuloma Angiotensin I-Converting Enzyme Activity by Regulatory T Lymphocytes in Murine Schistosomiasis

JOEL V. WEINSTOCK[1]* AND DOV L. BOROS[2]

Department of Medicine,[1] Division of Gastroenterology and Department of Immunology and Microbiology,[2] Wayne State University, Detroit, Michigan 48201

Received 11 August 1981/Accepted 20 October 1981

Granulomatous inflammatory lesions in murine schistosomiasis mansoni undergo a spontaneous diminution at the chronic phase of the disease concurrent with an increase in angiotensin I-converting enzyme (ACE) activity. The objective of this investigation was to determine whether T cells influence ACE activity within the granulomas. Cyclophosphamide and cimetidine treatments of mice, which augment delayed-type hypersensitivity reactions, enhanced liver granuloma size and decreased granuloma ACE activity. The suppression of liver granuloma by the adoptive transfer of spleen cells from chronically infected, immunomodulated mice into recipients with acute infection increased ACE activity of granulomas and granuloma macrophages. The increased ACE activity was dependent upon the transfer of Thy 1.2^+ splenic lymphocytes. The level of ACE activity was proportionate to the number of administered spleen cells. Thus, the level of ACE activity within the egg-induced granuloma is dependent upon T lymphocytes present in the spleen and granulomas of infected animals.

Murine schistosomiasis mansoni is a disease in which granulomas form around schistosome eggs disseminated in the liver and intestines (28). At the chronic stage of the infection, there is a spontaneous decrease in the size of newly generated granulomas in the liver and lung (1, 7). Modulation of the lesion size appears to be mediated by distinct subpopulations of T suppressor lymphocytes (2, 5, 8, 9, 30) that regulate inflammatory lymphokine production (4).

Previously, we detected angiotensin I-converting enzyme (ACE) activity in liver granulomas of schistosome-infected mice and showed that enzyme activity is associated with granuloma macrophages (29). Moreover, ACE specific activity markedly increased in the immunomodulated granulomas. The role of this enzyme in the granulomatous response is yet unelucidated. Increased serum ACE activity has been detected in some patients with granulomatous inflammation (14, 15, 22, 25) and is associated with the granuloma macrophage of sarcoidosis (E. Silverstein, J. Friedland, M. Drooker, C. Setton, and L. P. Perschuk, Clin. Res., p. 404, 1979).

The objective of this investigation was to further correlate elevated ACE activity with the immunoregulatory events that influence the granulomatous inflammation. Results indicate that the level of ACE activity within the granuloma may be influenced by splenic T suppressor lymphocytes.

MATERIALS AND METHODS

Animals used and methods of infection. Female CBA/J mice (Jackson Memorial Laboratories, Bar Harbor, Maine) 6 to 7 weeks of age were infected by subcutaneous injection of 25 cercariae of the Puerto Rican strain of *Schistosoma mansoni*. Perfusion of the portal veins of infected animals routinely yielded 8 to 10 worm pairs.

Determination of granuloma volume. Histological sections were made of multiple levels of granulomatous livers. Volumes of the lesions were calculated from the mean granuloma diameter, and diameters were measured by an automated image-splitting eyepiece (Vickers' Instrument, Inc., Woburn, Mass.) by determining the mean of two measurements made across the perpendicular axes. Only lesions with a single well-defined egg nidus were measured.

Spleen cell preparation. Spleen cell suspensions from mice infected for 20 weeks were prepared aseptically as described previously (3).

Spleen cell transfer regimen. A single injection of spleen cells from 20-week-infected mice was given intravenously in 0.5 ml of RPMI 1640 medium (Grand Island Biological Co., Grand Island, N.Y.) via the tail veins of 6-week-infected recipients. Dispersed spleen cells were >90% viable by trypan blue exclusion. Recipients were sacrificed at week 8 of infection, and livers were removed for histological preparation.

Antisera and complement treatment of spleen cells. Anti-Thy 1.2 serum (Bionetics Laboratories, Kensing-

ton, Md.) killed greater than 95% of the normal CBA/J thymocytes and less than 3% of the normal bone marrow cells. Rabbit serum absorbed with mouse tissues (Cedarlane Laboratories, London, Canada) served as the source of complement. Spleen cells were incubated for 30 min at 4°C in antiserum diluted 1:10 in RPMI medium (10^7 cells per ml). After incubation, the cells were washed by centrifugation at 4°C, suspended in an equal volume of a 1:10 dilution of complement, and then incubated again for 30 min at 37°C. Viability was determined by trypan blue staining.

Cyclophosphamide and cimetidine administration. Cyclophosphamide (Cytoxan; Mead Johnson & Co., Evansville, Ind.) was dissolved in sterile saline immediately before use and injected intraperitoneally. Animals received injections at a dose of 20 mg/kg at 6, 7, and 7.5 weeks of infection and were sacrificed at week 8.

Cimetidine HCl (Tagamet; Smith Kline & French Laboratories, Philadelphia, Pa.) was administered to animals in their drinking water at about 50 mg/kg per day from week 6 to 8 of infection. Drug recipients were sacrificed 8 weeks after infection. The drug was freshly dissolved in drinking water twice weekly. Animals were housed three to a cage.

Isolation of granulomas. Granulomas were isolated from the liver by the method of Pellegrino and Brener (cited in reference 6). Subsequently, the isolated granulomas were washed three times with Hanks balanced salt solution and then packed at 300 × *g* to remove supernatant.

Isolation and culture of granuloma cells. Isolated liver granulomas from mice infected for 8 weeks were placed in a 50-ml siliconized Erlenmeyer flask with RPMI 1640 medium (1 ml of medium per 0.5 ml of loosely packed tissue) containing 10% fetal calf serum (heat inactivated at 56°C for 30 min) and collagenase (1,600 u/ml) (both from Sigma Chemical Co., St. Louis, Mo.). The tissue suspension was agitated at 37°C in a shaker water bath for 50 min. The mixture was then centrifuged at 300 × *g* for 5 min to remove the collagenase solution and washed with RPMI medium. The loosened granulomas were dispersed by gentle teasing and pressing through a no. 80 stainless-steel screen. The dispersed cells were washed three times at 4°C with RPMI medium in siliconized glass test tubes. The yield was approximately 3×10^7 cells per liver, with 90% viability as determined by trypan blue exclusion. The dispersed cells comprised about 30% macrophages, 60% eosinophils, and 10% lymphocytes by morphological criteria.

The dispersed granuloma cells were suspended in RPMI medium containing 10% fetal calf serum, 2.0 mM glutamine, and 100 U of penicillin and 100 μg of streptomycin (Grand Island Biological Co., Grand Island, N.Y.) per ml. Cells (7×10^6 cells per 4 ml of medium) were cultured in 5-cm glass tissue culture dishes at 37°C in a 5% CO_2 atmosphere for 2 h. The nonadherent cells were removed from the monolayers by three washings. This fraction, which contained about 8% macrophage-like cells, 75% eosinophils, and 17% lymphocytes, was placed in 0.5 ml of 0.05 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 8.0), sonicated for 15 s on ice, and assayed for ACE activity.

The adherent cell population was >95% macrophage-like by morphological criteria. The cells were 10 to 30 μm in diameter, with oval eccentric nuclei. They displayed numerous vacuoles and pseudopodia. Some multinucleated giant cells were also seen. Ninety-five percent of these adherent cells phagocytized >10 latex beads per cell, and 80% were nonspecific esterase positive (29). The adherent cells were removed with a rubber policeman, suspended in 0.5 ml of 0.05 M HEPES buffer (pH 8.0), sonicated for 15 s on ice, and assayed for ACE activity.

The number of cells comprising the adherent cell fraction was determined by counting the number of nonadherent cells and subtracting this value from the total number of cells which were originally added to each dish.

Assay of ACE activity, protein, and DNA concentrations. All samples were stored for up to 1 month at −70°C for ACE activity protein, and DNA determinations. Under these conditions, there was no change in ACE activity.

ACE activity was determined by a radioassay method (19). The substrate was *p*-[^{3}H]hippuryl-glycylglycine (Ventrex Laboratories, Portland, Maine). ACE was extracted from granuloma tissues as previously described (29). Results were expressed as hippuric acid (nanomoles per minute) per milligram of protein or milligram of 2-deoxypentose in tissues and, in dispersed cells, as hippuric acid (nanomoles per minute) per 10^6 cells. Protein and 2-deoxypentose concentrations were measured by colorimetric methods (11). ACE activity, protein, and DNA determinations were performed on separate 200-mg samples of isolated liver granulomas.

Statistical methods. Data were subject to Student's *t* test to determine significant differences between groups. *P* values of <0.05 were considered significant.

RESULTS

Effect of alteration of granuloma size upon the specific activity of ACE. It was first determined whether granuloma size in the livers of acutely infected mice could be altered by adoptive cell transfer or by drugs. The drugs used were cyclophosphamide and cimetidine, which augment delayed-type hypersensitivity reactions, probably by impairment of suppressor T cell activity (16–18, 20, 26, 27). Adoptive transfer of 4×10^7 spleen cells from 20 week-infected mice into 6-week-infected recipients resulted in diminished liver granuloma size, after 2 weeks. In contrast, administration of cyclophosphamide intraperitoneally (20 mg/kg) at weeks 6, 7, and 7.5 of infection or of cimetidine orally (50 mg/kg per day) beginning at week 6 of infection resulted in the enhancement of liver granuloma size by week 8 of infection (Fig. 1A). There was no significant change in the concentration of DNA or of protein in isolated liver granulomas from 8-week-infected mice as a result of these manipulations. (Table 1).

We next determined the effect of these treatments upon ACE specific activity in isolated liver granulomas of acutely infected animals. The transfer of 4×10^7 spleen cells from 20-

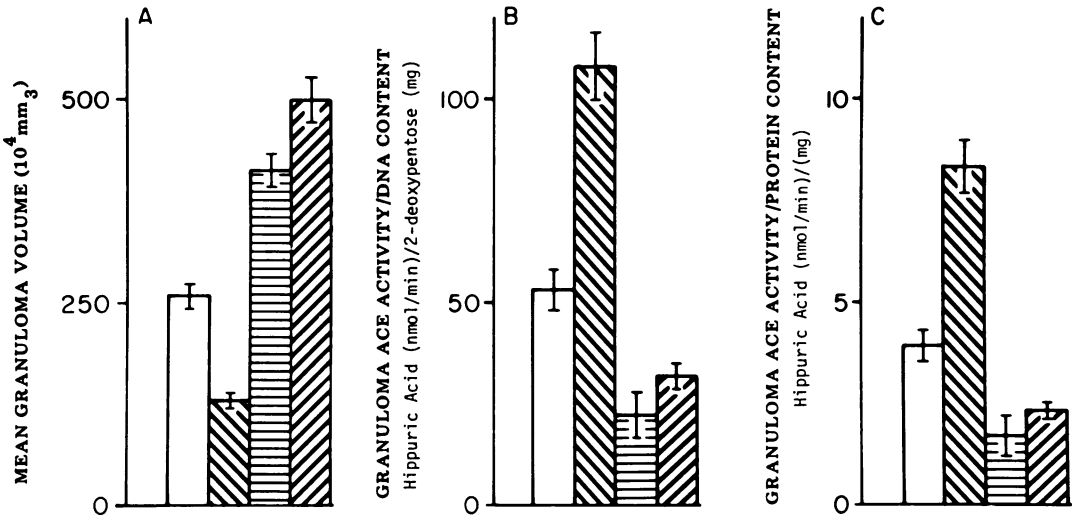

FIG. 1. Size and ACE specific activity of liver granulomas from acutely infected mice after adoptive transfer of 4×10^7 spleen cells from chronically infected animals or treatment with either cyclophosphamide or cimetidine. Volume and ACE activity data are mean values of two experiments from six mice ± standard error of the mean. Volume data for each mouse were derived from 20 observations. Symbols: □, no treatment; ▨, chronic spleen cells, ▤, cyclophosphamide; and ▨, cimetidine.

week-infected mice enhanced, whereas the treatment of mice with cyclophosphamide or cimetidine diminished, the specific activity of ACE within granulomas. This trend was similar whether values were calculated on the basis of DNA or on protein content (Fig. 1B and C). Moreover, the incremental changes in both granuloma size and ACE specific activity were proportionate to the number of splenic cells transferred (Fig. 2).

Treatment of spleen cells with anti-Thy 1.2 plus complement. To further identify the cell responsible for the elevated ACE activity in adoptive transfer, spleen cells were treated with anti-Thy 1.2 plus complement or with normal mouse serum plus complement. Anti-Thy 1.2 treatment of spleen cells before transfer eliminated the ability of the cells to alter both the specific activity of ACE and the granuloma size (Fig. 3).

Effect of adoptive transfer on ACE activity associated with granuloma macrophages. Dispersed liver granuloma cells from acutely infected animals which received 4.0×10^7 spleen cells from 20-week-infected mice had increased ACE activity. This increased activity was associated with the adherent cell fraction and comprised predominately macrophages (Table 2).

DISCUSSION

Previous studies have shown that the spontaneously modulated granulomatous response that occurs in mice chronically infected with *S. mansoni* (1, 7) is regulated by T lymphocytes (2, 4, 5, 8, 9, 30). We recently showed that the diminished granulomatous response in mice undergoing modulation is associated with high ACE activity in the serum, liver, and colon granulomas (29). In the present study, we further explored the relationship between ACE activity and the immunoregulation of the granulomatous response.

Results showed that immunomanipulations which caused diminished or increased granulomatous responses resulted in an inverse alteration of ACE activity within the lesions. Despite significant differences in granuloma sizes, no change was measured in the DNA and protein concentration of the homogenized lesions. Since a unit wet weight of packed tissue would be expected to contain more small than large granu-

TABLE 1. Effect of adoptive cell transfer and drug treatments on granuloma DNA and protein concentrations

Treatment	DNA content/ granuloma wet wt (2-deoxypentose [mg]/g)	Protein content/ granuloma wet wt (mg/g)
None	2.54 ± 0.10[a]	34.5 ± 1.5[a]
Chronic spleen cells	2.32 ± 0.05	32.9 ± 0.5
Cyclophosphamide	2.24 ± 0.08	31.7 ± 0.6
Cimetidine	2.40 ± 0.01	28.7 ± 1.5

[a] Control versus treatments; *P* not significant; data are mean values ± SEM of six experiments.

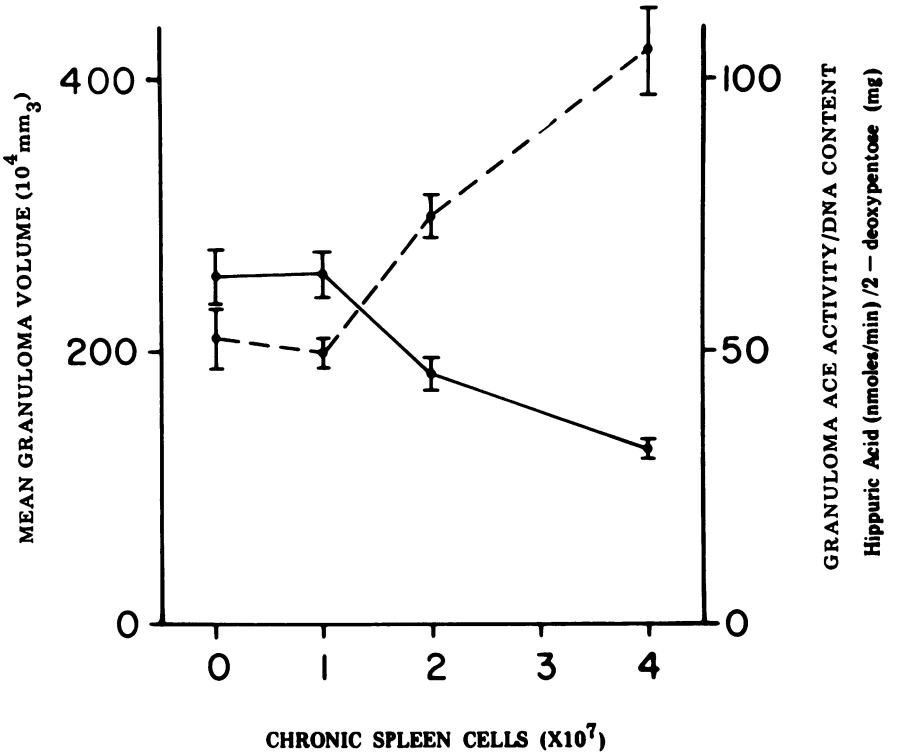

FIG. 2. Size and ACE specific activity of liver granulomas from acutely infected mice after adoptive transfer of graded doses of spleen cells from chronically infected animals. Volume and ACE activity data are mean values of two experiments from six mice ± standard error of the mean. Volume data for each mouse were derived from 20 observations. Symbols: ———, volume; ----, ACE activity.

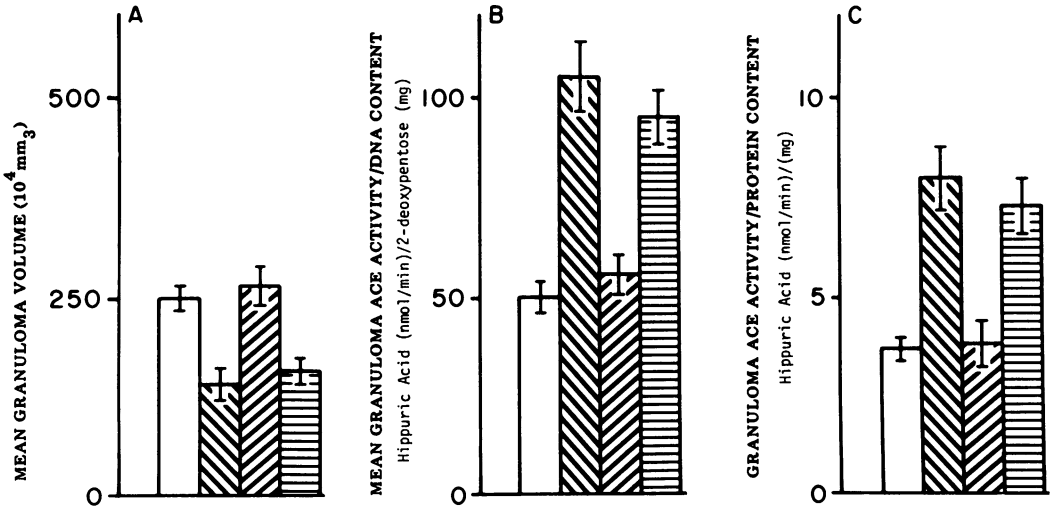

FIG. 3. Characterization of spleen cells which adoptively suppress granuloma size and increase ACE specific activity by anti-Thy 1.2 antisera and complement treatment. Volume and ACE activity data are mean values of two experiments from six mice ± standard error of the mean. Volume data for each mouse were derived from 20 observations. Symbols: □, no treatment; ▨, chronic spleen cells; ▨, chronic spleen cells treated with anti-Thy 1.2 plus complement; and ▤, chronic spleen cells treated with normal mouse serum plus complement.

TABLE 2. ACE activity of dispersed liver granuloma cells from acutely infected animals after adoptive transfer of spleen cells from chronically infected donors

Treatment	ACE activity (nmol/min) per 10^6 cells		
	Unfractionated granuloma cells	Adherent cell fraction	Nonadherent cell fraction
Cell transfer	0.491 ± 0.280[a]	1.170 ± 0.161	0.180 ± 0.140
No transfer	0.129 ± 0.042	0.360 ± 0.113	0

[a] Data are mean values ± SD of six separate experiments. Each experiment utilized the granulomas from two to three livers.

lomas, the smaller modulated lesions should contain fewer cells. This suggests that immunomanipulation of the lesions resulted in inverse alterations of ACE activity and cell content.

Enzyme activity was located in the adherent cell fraction of the granuloma cells, strongly suggesting that macrophages produce ACE. Enzyme production by macrophages was strongly influenced by T suppressor lymphocytes because the adoptive transfer of such cells not only diminished the vigorous granulomatous responses of the recipients, but also caused an increase in ACE activity of the granuloma macrophages.

ACE appears to be an inducible enzyme of the granuloma mononuclear phagocyte. Evidence suggesting inducibility includes the observation that only some granulomatous inflammations are associated with abundant ACE activity (15, 25). Furthermore, synthesis of ACE in cultures of rabbit alveolar macrophages and human peripheral blood monocytes has been induced by corticosteroids and stimulated peripheral lymphocytes (23).

Although our present results do not indicate de novo induction of ACE production, they strongly suggest that enzyme activity is under the influence of T suppressor lymphocytes. Cyclophosphamide and cimetidine have been shown to enhance delayed hypersensitive responses by the elimination of T suppressor cells or by blockage of their H_2 receptors (16–18, 20, 26, 27). These drugs indeed augmented granulomatous responses in the drug-treated mice. The concomitant decrease of granuloma ACE levels in treated animals illustrates the close relationship between enzyme activity and the presumed local effect of T suppressor cells. This is also supported by the observed effect of adoptive transfer on granuloma size and ACE activity.

Ongoing analysis of the T cell circuitry active during the egg-specific granulomatous response shows that the inflammation is generated by lymphokine-secreting Ly 1^+ delayed hypersensitivity T cells (4, S. R. Wellhausen and D. L. Boros, Fed. Proc., p. 1002, 1981) which are regulated by a cyclophosphamide-sensitive Ly $2, 3^+$ subset of T suppressor lymphocytes (5, 9). In the present experiments, the comparable efficacy of cimetidine treatment in the enhancement of the granulomatous response seems to indicate that T suppressor lymphocytes which regulate delayed hypersensitive T cell activity may also bear H_2 receptors on their surfaces. This is consonant with previous studies showing that guinea pig lymphocytes with histamine receptors can suppress migration inhibition factor production (17). This finding is of special interest in the light of recent observations which show that mast cells are present in the liver granulomas of normal littermates, but not in athymic nude mice (11).

Although high ACE activity has been found in the serum, granulomatous lymph nodes, and macrophages of patients with sarcoidosis and other granulomatous diseases (14, 15, 22, 25), the functional significance of this enzyme in the granulomatous inflammatory process is unknown. ACE converts angiotensin I to angiotensin II and inactivates bradykinin (24). The presence of angiotensins has not yet been sought in granulomatous inflammations. They could theoretically alter inflammatory responses by stimulation of prostaglandin release (21), or by directly affecting contractile elements associated with the cytoskeleton of inflammatory cells (10). In addition, angiotensins could affect the microvasculature of the granuloma, thus influencing blood supply and cellular egression from vessels. Similarly, ACE inactivation of kinins, presumably participating in the schistosome egg-induced granulomas (13), could modify the granulomatous response. However, it is still to be determined whether granuloma ACE is important in the inflammatory response, and if so, whether it is acting upon these or some other as yet unidentified mediator involved in the inflammatory process.

ACKNOWLEDGMENTS

We thank Arthur Blum for his excellent technical assistance and Harriet Anne Nolan for invaluable help in the preparation of this manuscript.

This research was supported by Public Health Service grant AI-12913 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Boros, D. L., R. P. Pelley, and K. S. Warren.** 1975. Spontaneous modulation of granulomatous hypersensitivity in schistosomiasis mansoni. J. Immunol. **114**:1437–1441.
2. **Chensue, S. W., and D. L. Boros.** 1979. Modulation of granulomatous hypersensitivity. I. Characterization of T lymphocytes involved in the adoptive suppression of granuloma formation in *Schistosoma mansoni*-infected mice. J. Immunol. **123**:1409–1414.
3. **Chensue, S. W., and D. L. Boros.** 1979. Population dynamics of T and B lymphocytes in the lymphoid organs, circulation, and granulomas of mice infected with *Schistosoma mansoni*. Am. J. Trop. Med. Hyg. **28**:291–299.
4. **Chensue, S. W., D. L. Boros, and C. S. David.** 1980. Regulation of granulomatous inflammation in murine schistosomiasis. In vitro characterization of T lymphocyte subsets involved in the production and suppression of migration inhibition factor. J. Exp. Med. **151**:1398–1412.
5. **Chensue, S. W., S. R. Wellhansen, and D. L. Boros.** 1981. Modulation of granulomatous hypersensitivity. II. Participation of Ly 1^+ and Ly 2^+ T lymphocytes in the suppression of granuloma formation and lymphokine production in *Schistosome mansoni*-infected mice. J. Immunol. **127**:363–367.
6. **Coker, C. M., and F. Lichtenberg.** 1956. Revised method for isolation of *Schistosoma mansoni* eggs for biological experimentation. Proc. Soc. Exp. Biol. Med. **92**:780–782.
7. **Colley, D. G.** 1975. Immune responses to a soluble schistosomal egg antigen preparation during chronic primary infection with *Schistosoma mansoni*. J. Immunol. **115**:150–156.
8. **Colley, D. G.** 1976. Adoptive suppression of granuloma formation. J. Exp. Med. **143**:696–700.
9. **Colley, D. G., F. A. Lewis, and C. W. Todd.** 1979. Adoptive suppression of granuloma formation by T lymphocytes and by lymphoid cells sensitive to cyclophosphamide. Cell. Immunol. **46**:192–200.
10. **Dezsö, B., and G. Fóris.** 1981. Effect of angiotensin II on the Fc receptor activity of rat macrophages. Immunology **42**:277–283.
11. **Epstein, W. L., K. Fukuyama, K. Danno, and E. Kwan-Wong.** 1979. Granulomatous inflammation in normal and athymic mice infected with schistosoma mansoni: an ultrastructural study. J. Pathol. **127**:207–215.
12. **Keleti, G., and W. H. Lederer.** 1974. Handbook of micromethods for the biological sciences. Van Nostrand Reinhold Company, New York.
13. **Kellermeyer, R. W., and K. S. Warren.** 1970. The role of chemical mediators in the inflammatory response induced by foreign bodies: comparison with the schistosome egg granuloma. J. Exp. Med. **131**:21–39.
14. **Lieberman, J.** 1975. Elevation of serum angiotensin-converting enzyme (ACE) level in sarcoidosis. Am. J. Med. **59**:365–372.
15. **Lieberman, J., A. Nosal, L. Schlessner, and A. Sastre-Foken.** 1979. Serum angiotensin-converting enzyme for diagnosis and therapeutic evaluation of sarcoidosis. Am. Rev. Respir. Dis. **120**:329–335.
16. **Plaut, M., L. M. Lichtenstein, and C. S. Henney.** 1975. Properties of a subpopulation of T cells bearing histamine receptors. J. Clin. Invest. **55**:856–874.
17. **Rocklin, R. E.** 1977. Histamine-induced suppressor factor (HSF): effect on migration inhibitory factor (MIF) production and proliferation. J. Immunol. **118**:1734–1738.
18. **Rocklin, R. E., D. K. Greineder, and K. L. Melmon.** 1979. Histamine-induced suppressor factor (HSF): further studies on the nature of the stimulus and the cell which produces it. Cell. Immunol. **44**:405–415.
19. **Ryan, J. W., A. Chung, C. Ammons, and M. L. Carlton.** 1977. A simple radioassay for angiotensin-converting enzyme. Biochem. J. **167**:501–504.
20. **Schwartz, A., P. W. Askenase, and R. K. Gershon.** 1978. Regulation of delayed-type hypersensitivity reactions by cyclophosphamide-sensitive T cells. J. Immunol. **121**:1573–1577.
21. **Shibouta, Y., Y. Inada, Z. Terashita, K. Nishikawa, S. Kikuchi, and K. Shimamoto.** 1979. Angiotensin-II-stimulated release of thromboxane A2 and prostacyclin (PG12) in isolated, perfused kidneys of spontaneously hypertensive rats. Biochem. Pharmacol. **28**:3601–3609.
22. **Silverstein, E., J. Friedland, H. A. Lyons, and A. Gourin.** 1976. Elevation of angiotensin-converting enzyme in granulomatous lymph nodes and serum in sarcoidosis: clinical and possible pathogenic significance. Ann. N.Y. Acad. Sci. **278**:498–513.
23. **Silverstein, E., J. Friedland, and C. Setton.** 1979. Angiotensin converting enzyme: induction in rabbit alveolar macrophages and human monocytes in culture. Adv. Exp. Med. Biol. **121**:149–156.
24. **Soffer, R. L.** 1976. Angiotensin-converting enzyme and the regulation of vasoactive peptides. Annu. Rev. Biochem. **45**:73–94.
25. **Studdy, P., R. Bird, D. G. James, and S. Sherlock.** 1978. Serum angiotensin-converting enzyme (SACE) in sarcoidosis and other granulomatous disorders. Lancet **ii**:1331–1334.
26. **Sy, M. S., S. D. Miller, and H. N. Claman.** 1977. Immune suppression with supraoptimal doses of antigen in contact sensitivity. I. Demonstration of suppressor cells and their sensitivity to cyclophosphamide. J. Immunol. **119**:240–244.
27. **Van Dijk, H., M. Rapis, H. E. Jacobse-Geels, and J. M. N. Willers.** 1979. Histamine 2 receptor-mediated immunomodulation in the mouse. I. Immunomodulation by the H_2 agonist tolazoline. Clin. Exp. Immunol. **35**:470–477.
28. **Warren, K. A.** 1980. The relevance of schistosomiasis. N. Engl. J. Med. **303**:203–206.
29. **Weinstock, J. V., D. L. Boros, and J. B. Gee.** 1981. Enhanced granuloma angiotensin I converting enzyme activity associated with modulation in murine schistosomiasis. Gastroenterology **81**:48–53.
30. **Wellhausen, S. R., S. W. Chensue, and D. L. Boros.** 1980. Modulation of granulomatous hypersensitivity: analysis by adoptive transfer of effector and suppressor T lymphocytes involved in granulomatous inflammation in murine schistosomiasis, p. 219–234. *In* D. L. Boros and T. Yoshida (ed.), Basic and clinical aspects of granulomatous diseases. Elsevier/North Holland, Publishing Co., New York.