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Immunoregulation in Disseminated Histoplasmosis: Characterization of the Surface Phenotype of Splenic Suppressor T Lymphocytes

SUSAN R. WATSON* AND WARD E. BULLOCK

Division of Infectious Diseases, Department of Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267

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C57BL/6 and C3H/HeJ mice were infected intravenously with 6×10^5 yeast-phase *Histoplasma capsulatum* organisms. After 1 week, splenocytes from both mouse strains showed diminished antibody responses to sheep erythrocytes in vitro; these cells also were able to suppress the response of normal syngeneic cells. Passage of splenocytes from infected mice through a nylon wool column yielded a population enriched for T cells that exerted suppressor activity, although to a smaller extent than did unfractionated cells. Treatment of the T-cell-enriched population from both strains of mice with either anti-Thy 1.2 or anti-Ly 2 and complement resulted in a loss of this immunosuppressive ability. In addition, anti-I-J^k antiserum was effective in ablating the suppressive effect of C3H/HeJ nylon wool-passed spleen cells. The conclusion drawn from these experiments is that the T cells from *H. capsulatum*-infected animals which are capable of modulating the in vitro plaque-forming cell response to sheep erythrocytes bear Ly 2 and I-J determinants on their surfaces.

In the last decade, the complexity of cellular interactions involved in mounting and modulating an immune response has become apparent (5). Studies investigating these interactions in mice, and more recently in humans, have been facilitated by the discovery of T-cell surface antigens that permit correlation between the phenotype and the functional repertoire of the T-cell subclasses. Cantor and Boyse (3) have defined the Ly antigen system in mice. Helper T cells are characterized by the expression of Ly 1 antigen but not of Ly 2 or Ly 3 antigen (Ly $1^+,23^-$). These cells have been shown to help in the differentiation and expansion of cytotoxic T cells and to provide helper functions for T-cell-dependent B-cell responses (6). The other major group of T cells is distinguished by the phenotype Ly $1^-,23^+$ and contains cells capable of both suppressor activity and the generation of cytotoxic responses (4). To date, the only means by which to separate these two activities phenotypically is based on I-region gene products. Suppressor cells have been defined as Ly $1^-,23^+$, I-J^+, whereas cytotoxic T cells are Ly $1^-,23^+$,I-J^- (19).

Our laboratory has been interested in the disturbances of the immune system accompanying disseminated granulomatous infections, and the infection caused by the biphasic fungus *Histoplasma capsulatum* has been of particular interest. To investigate the possible complexities involved in the human disease state, a murine model of disseminated histoplasmosis has been established. Intravenous injection of C3H/AnF mice with yeast-phase organisms results in a disseminated infection that resolves without therapeutic intervention over an 8- to 10-week period (1). In previous studies, we have shown that from 1 to 3 weeks after infection, the delayed-type hypersensitivity response to *Histoplasma* antigens is depressed, as is the delayed-type hypersensitivity response to sheep erythrocytes (SRBC). In vitro blastogenic responses to both specific antigen (histoplasmin) and a nonspecific mitogen (concanavalin A) of spleen cells from these animals are depressed as compared with age-matched control mice (2). Moreover, spleen cells from infected animals consistently suppress both the T-cell-dependent antibody response to SRBC by syngeneic splenocytes in vitro and the T-cell-mediated cytotoxicity responses of normal syngeneic cells (2). Additional studies (20) have demonstrated the presence of both suppressor T cells and macrophage-like suppressor cells in the spleens of mice during the anergic phase of infection (1 to 5 weeks).

In this communication, we present the results of studies performed to define more clearly the surface phenotype of the suppressor T cells involved in disseminated *Histoplasma* infection.

The suppressor cell population was investigated for the Ly phenotype and the presence or absence of I-J determinants on their surfaces.

MATERIALS AND METHODS

Animals and preparation of yeasts. Male C3H/HeJ (H-2^k:Ly 1.1:2.1:3.2) or C57BL/6 (H-2^b:Ly 1.2:2.2:3.2) mice (Jackson Laboratory, Bar Harbor, Maine), 6 to 8 weeks of age, were infected intravenously with 0.5×10^6 to 1×10^6 yeast-phase *H. capsulatum* organisms of strain G-217-B. Inocula were prepared by harvesting yeast-phase organisms after 36 h of growth in brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) at a gyratory shaking speed of 175 rpm. Yeast cells were washed three times with Hanks balanced salt solution. After a final centrifugation at $30 \times g$ to remove larger yeast aggregates, the organisms were counted and diluted in Hanks balanced salt solution to the final concentration before injection.

Spleen cell preparation, cell culture, and PFC assay. Single spleen cells were prepared by gently teasing the organ between two ground glass slides in Hanks balanced salt solution. Tissue debris was removed by sedimentation at $1 \times g$, and the cells were washed twice in Hanks balanced salt solution. The resulting cells (viability > 92% as determined by trypan blue dye exclusion) were cultured as described by Mishell and Dutton (17) in the presence of 0.1% SRBC as an antigen; SRBC were obtained from a single sheep every 3 to 4 weeks (Colorado Serum Co., Denver, Colo.). After 4 days, the cultured cells were assayed for the presence of 19S (immunoglobulin M [IgM]) antibody plaque-forming cells (PFC) by the Cunningham-Szenberg modification of the Jerne technique (9). Responses are expressed as the mean number of PFC per 10^6 cells recovered ± standard error of the mean as calculated from quadruplicate cultures.

Nylon wool separation of spleen cells. Spleen cells were fractionated on nylon wool columns by the method of Julius et al. (14).

Antisera employed. (i) Monoclonal anti-Thy 1.2. Monoclonal anti-Thy 1.2 was obtained in the form of the supernatant from cultures of HO-13.4.9 hybridoma cells. This antibody is of the IgM class and in the presence of complement is lytic for T cells. A full analysis of the antibody has been published (16).

(ii) Anti-Ly 1. Anti-Ly 1.1 was purchased from Accurate Scientific, Hicksville, N.Y. This monoclonal antibody is of the mouse IgG2a class and fixes complement directly. Anti-Ly 1.2 was purchased as an alloantiserum from Accurate Scientific. Monoclonal anti-Ly 1 was prepared as the supernatant from cultures of the hybridoma line 53.7.313. This monoclonal antibody is a rat IgG2a that fixes complement with various efficiencies depending on antigen density and recognizes only the framework determinants of the Ly 1 antigens. An analysis of the immunoglobulin product from the 53.7.313 hybridoma cell line has been published (15).

(iii) Anti-Ly 2. Anti-Ly 2.1 was purchased from Accurate Scientific. This is a cytotoxic monoclonal antibody of the mouse IgG3 subclass. Anti-Ly 2.2, an alloantiserum, was obtained from Accurate Scientific. Monoclonal anti-Ly 2 was prepared as the supernatant from cultures of the hybridoma line 53.6.72. This antibody is a rat IgG2a and does not distinguish between the allelic forms of the Ly 2 antigen; it fixes complement with various efficiencies depending on antigen density (15).

(iv) Anti-I-J^k. Anti-I-J^k was purchased as an alloantiserum from Accurate Scientific.

(v) Anti-rat IgG. Anti-rat IgG was purchased from Cappel Laboratories, West Chester, Pa.

All hybridoma cell lines were obtained from the Cell Distribution Center, The Salk Institute, La Jolla, Calif.

Antiserum treatment. All antisera from Accurate Scientific were used at the working concentrations specified by the manufacturer in its technical information sheets. Hybridoma supernatants prepared in our laboratory were used at a 10^{-1} dilution. Nylon wool-passed splenic T cells (>95% T cells by immunofluorescence) were mixed with the appropriate antiserum at a concentration of 2×10^7 cells per ml at 4°C for 30 min. The cells were washed twice with ice-cold medium (RPMI 1640 plus 5% fetal calf serum), incubated with a 1/10 dilution of Lox Tox rabbit complement (Accurate Scientific) at 37°C for 30 min, washed twice again, and then used in the appropriate experiment. In experiments with rat monoclonal anti-Ly antibodies, cells that had been incubated with these antibodies were washed twice in ice-cold medium and then incubated with a 1/10 dilution of anti-rat IgG for 30 min at 4°C. The cells were again washed in ice-cold medium and incubated with complement as described above.

RESULTS

Generation of splenic suppressor T-cell populations by both C57BL/6 and C3H/HeJ mice. As most of our previous work on the murine model of histoplasmosis had employed C3H/AnF mice, an initial experiment was performed to determine whether both C3H/HeJ and C57BL/6 mice developed splenic suppressor T cells 1 week after infection. The primary antibody responses to SRBC were measured in cultures containing the following cells: (i) unfractionated spleen cells from normal mice; (ii) spleen cells from infected mice; (iii) mixtures of these cells; (iv) T cells from normal mice cocultured with normal splenocytes; and (v) T cells from infected mice cocultured with normal splenocytes. The PFC responses to SRBC by spleen cells from both strains of mice were suppressed at 1 week after infection (Table 1). Cells from these mice also depressed the responses of normal syngeneic cells. When nylon wool-passed spleen cells from infected animals were cocultured with normal unfractionated syngeneic spleen cells, they suppressed the in vitro PFC response.

Several points should be emphasized regarding these experiments. First, the addition of normal T cells to normal unfractionated spleen cells consistently increased the percentage of viable cells recovered from the cultures (46% as compared with 25%). Therefore, the effect of T cells from infected mice on the normal response was calculated as a percentage of the PFC response of normal spleen cells cultured with

TABLE 1. Primary antibody response to SRBC by spleen cell cultures from C57BL/6 and C3H/HeJ mice

Cell source	PFC response per 10^6 cells recovered ± SEM			
	C57BL/6 mice		C3H/HeJ mice	
	Expt 1	Expt 2	Expt 1	Expt 2
Normal spleen[a]	12,894 ± 1,431	6,048 ± 500	1,952 ± 126	1,014 ± 94
Infected spleen[a]	53 ± 23 (0.04)[b]	100 ± 43 (1.6)	26 ± 11 (1.3)	33 ± 14 (3.2)
Normal + infected spleen[c]	2,624 ± 29 (20.4)	2,400 ± 279 (39.7)	150 ± 29 (7.6)	165 ± 4 (16.0)
Normal spleen + normal T[d]	10,100 ± 376	5,493 ± 533	1,472 ± 86	1,206 ± 73
Normal spleen + infected T	4,809 ± 634 (47.6)[e]	3,666 ± 226 (66.7)	466 ± 14 (31.7)	644 ± 51 (53.4)

[a] Each culture contained 1.5×10^7 spleen cells from a pool of five or more mice.
[b] Numbers in parentheses show percentage of normal response.
[c] Each culture contained 1×10^7 normal spleen cells and 5×10^6 spleen cells from infected mice.
[d] Each culture contained 1×10^7 normal spleen cells and 5×10^6 nylon wool-passed T cells.
[e] Percentage of normal response calculated as percentage of the response of normal spleen cells plus normal T cells; these experiments are representative of six similar studies.

normal T cells after all the counts had been corrected to PFC per 10^6 cells recovered from the cultures. Second, splenocytes from normal C57BL/6 mice responded much better in vitro to SRBC than did C3H/HeJ cells, as is characteristic of the PFC responses of these two strains. Finally, it was clear that nylon wool-passed cells from infected animals did not suppress the response of normal cells as severely as did unfractionated spleen cells. This finding is explained by the fact that nylon wool passage of splenocytes removed a macrophage-like cell that exerts a suppressor activity in murine histoplasmosis (20).

These initial experiments demonstrated that, as with C3H/AnF mice (21), splenocytes from both C57BL/6 and C3H/HeJ mice showed depressed PFC responses to SRBC at 1 week after systemic infection with yeast-phase *H. capsulatum*. Moreover, both unfractionated and nylon wool-passed cells from infected mice suppressed the response of normal syngeneic spleen cells.

Ly phenotype of splenic suppressor T cells. Two sets of experiments were performed to determine the Ly phenotype of the nylon-wool-passed cells from infected animals. The first set employed monoclonal anti-Ly antisera that recognized only the framework determinants of the Ly antigens, and cells from either C57BL/6 or C3H/HeJ mice (Table 2). The ability of nylon wool-passed splenocytes to suppress the PFC response of normal unfractionated spleen cells was largely ablated by treatment with either anti-Thy 1.2 or anti-Ly 2, but not with anti-Ly 1.

TABLE 2. Effect of various monoclonal antisera on suppression of the normal splenocyte PFC response by splenic T cells from *H. capsulatum*-infected C57BL/6 and C3H/HeJ mice

Mouse strain	Antiserum treatment of T cells	PFC per 10^6 cells recovered ± SEM			
		Expt 1		Expt 2	
		Normal mice[a]	Infected mice[b]	Normal mice[a]	Infected mice[b]
C57BL/6	None	8,084 ± 196	4,040 ± 341 (50.0)[c]	13,119 ± 991	5,447 ± 1,259 (41.1)
	Anti-Thy 1.2 + C[d]	7,846 ± 532	6,983 ± 549 (89.0)	10,047 ± 763	9,042 ± 687 (90.0)
	Anti-Ly 1 + C	8,001 ± 149	2,640 ± 849 (33.3)	7,952 ± 120	3,180 ± 248 (40.0)
	Anti-Ly 2 + C	8,434 ± 377	7,759 ± 811 (91.9)	8,190 ± 1,222	7,699 ± 1,149 (94.0)
	C alone	8,266 ± 647	3,471 ± 406 (42.2)	8,095 ± 756	4,452 ± 309 (55.0)
C3H/HeJ	None	1,283 ± 94	211 ± 14 (16.4)	849 ± 91	297 ± 46 (35.0)
	Anti-Thy 1.2 + C	1,196 ± 201	1,231 ± 314 (103.0)	901 ± 104	847 ± 95 (94.0)
	Anti-Ly 1 + C	938 ± 81	225 ± 39 (24.0)	1,016 ± 26	406 ± 31 (40.0)
	Anti-Ly 2 + C	1,024 ± 111	911 ± 86 (89.0)	894 ± 94	797 ± 83 (89.1)
	C alone	1,135 ± 89	177 ± 24 (15.6)	1,004 ± 131	412 ± 39 (41.2)

[a] Each culture contained 5×10^6 T cells from normal animals added to 1×10^7 normal splenocytes from five or more mice. The T cells were treated with various antisera as specified.
[b] Each culture contained 5×10^6 T cells from infected animals added to 1×10^7 normal splenocytes. The T cells were treated with various antisera as specified.
[c] Numbers in parentheses show percentage of normal response calculated as percentage of the response of 1×10^7 normal splenocytes plus 5×10^6 normal T cells after various antiserum treatments; these experiments are representative of five similar studies.
[d] C, Complement.

TABLE 3. Confirmation of Ly phenotype of suppressor T cells in *H. capsulatum*-infected C57BL/6 mice by allele-specific Ly antisera

Treatment of nylon wool-passed T cells	PFC per 10^6 cells recovered ± SEM			
	Expt 1		Expt 2	
	Normal mice[a]	Infected mice[b]	Normal mice[a]	Infected mice[b]
None	5,608 ± 489	3,230 ± 148 (57.6)[c]	7,189 ± 806	3,465 ± 517 (48.2)
Anti-Ly 1.1 + C[d]	6,186 ± 304	2,555 ± 311 (41.3)	8,004 ± 1,206	3,121 ± 149 (39.0)
Anti-Ly 1.2 + C	5,714 ± 716	2,514 ± 408 (44.0)	7,883 ± 642	2,814 ± 346 (35.4)
Anti-Ly 2.1 + C	5,230 ± 812	2,244 ± 377 (42.9)	7,697 ± 546	3,733 ± 418 (48.5)
Anti-Ly 2.2 + C	6,304 ± 517	5,207 ± 486 (82.6)	8,109 ± 809	7,689 ± 994 (89.9)

[a] See Table 2, footnote *a*.
[b] See Table 2, footnote *b*.
[c] See Table 2, footnote *c*. These experiments are representative of four similar studies.
[d] C, Complement.

To substantiate these findings, anti-Ly antisera were used that recognized the allele-specific determinants of the Ly antigens. This series of experiments was performed with splenocytes from C57BL/6 mice. The results (Table 3) demonstrate that only anti-Ly 2.2 abrogated the suppressive effect of T cells from C57BL/6 mice, thus confirming the findings of the previous experiment.

I-J phenotype of suppressor T cells. Currently, the only means of distinguishing suppressor Ly 2^+ cells from cytotoxic Ly 2^+ cells is by the presence of I-J determinants on the suppressor T cells (19). Therefore, nylon wool-passed splenocytes from C3H/HeJ mice infected 1 week previously were treated with anti-I-J^k antiserum and complement to determine whether these cells bore the I-J determinant. This experiment was performed with cells from C3H/HeJ mice since the only commercially available I-J antiserum is anti-I-J^k. The results (Table 4) indicated that treatment with anti-I-J^k antiserum removed the suppressive effect of nylon wool-passed splenic T cells from C3H/HeJ mice.

DISCUSSION

Results presented in this paper indicate that the suppressive effect of T cells from *H. capsulatum*-infected C3H/HeJ and C57BL/6 mice on normal syngeneic splenocytes is abrogated by treatment with anti-Ly 2 antiserum and complement. In the case of C3H/HeJ splenic T cells, anti-I-J^k antiserum was also effective. The residual suppressive effect of T cells from infected mice after treatment with anti-Ly 2 and complement varied from 6 to 18%. It is possible that this variation is a reflection of the fact that the efficiency of complement binding to the rat monoclonal antibodies employed is dependent on antigen density (15) and that T cells from infected animals express less Ly 2 upon their cell surfaces (S. R. Watson et al., manuscript in preparation). Therefore, it is possible that in some experiments, cells possessing very few Ly 2 antigenic determinants were not killed and hence were still able to modulate the in vitro response.

We conclude from these experiments that, at least in C3H/HeJ mice, both Ly 2^+ T cells and I-J^+ cells are required to suppress the PFC response of normal splenocytes. In addition, it can be postulated that cells bearing the same phenotypic markers exert suppressive activity in the C57BL/6 system, although we were not able to obtain data regarding the I-J phenotype of these cells due to the lack of a readily available anti-I-

TABLE 4. Presence of I-J determinants on suppressor T cells from C3H/HeJ mice

Treatment of nylon wool-passed T cells	PFC per 10^6 cells recovered ± SEM			
	Expt 1		Expt 2	
	Normal mice[a]	Infected mice[b]	Normal mice[a]	Infected mice[b]
None	1,923 ± 160	784 ± 86 (40.8)[c]	2,804 ± 56	904 ± 101 (32.2)
Anti-Thy 1.2 + C[d]	2,500 ± 80	2,385 ± 78 (95.4)	2,120 ± 168	1,994 ± 203 (94.1)
Anti-I-J^k + C	2,667 ± 80	2,539 ± 98 (95.2)	2,640 ± 146	2,319 ± 219 (87.8)
C alone	2,130 ± 240	690 ± 42 (32.4)	3,009 ± 201	1,124 ± 142 (37.4)

[a] See Table 2, footnote *a*.
[b] See Table 2, footnote *b*.
[c] See Table 2, footnote *c*. These experiments are representative of three similar studies.
[d] C, Complement.

J^b antiserum. Whether these two surface markers are borne on the same subpopulation of T cells is unknown. Initial experiments in which positive selection procedures such as cell affinity chromatography (21) were used yielded populations of cells from infected animals insufficiently purified to give consistent results (S. R. Watson, unpublished data). However, these experiments will be repeated with a fluorescence-activated cell sorter. This should allow us to obtain populations of cells of 99% purity, and this may make more conclusive results possible. Also unknown at this time is the ultimate cellular target of the suppressor cell(s). Studies are being undertaken to investigate this aspect of the system.

In normal mice much is known about the interactions of various T-cell subsets (as defined by anti-Ly antiserum) in the regulation of the immune response (10, 12). In addition, the discovery of the *I-J* subregion of the mouse major histocompatibility (*H-2*) complex has allowed further clarification of these subsets. Determinants coded for by the *I-J* subregion of the mouse *H-2* complex have been found to be expressed on cells involved in the generation of specific suppressor T cells, on a subset of helper T cells, and on macrophages required for the development of in vitro antibody responses (18). Soluble factors mediating the generation of both suppressor and helper activities also have been shown to possess such antigenic determinants (11).

Although many groups have identified suppressor T cells in infectious disease models, little work has been done to determine the Ly and I-J phenotype of these cells. Recently, however, Green and Colley (13) have studied the role of cells bearing these respective phenotypes in the modulation of egg-induced granuloma formation in mice experimentally infected with *Schistosoma mansoni*. By means of adoptive transfer of T cells between congenic mouse strains that differed only at the *I-J* locus, these workers demonstrated that the modulation of granuloma formation was I-J restricted. They further determined that granuloma modulation was sensitive to in vivo treatment with microliter amounts of anti-I-J antiserum. Concurrently, Chensue and Boros (7) determined that adoptive T-cell modulation of egg-induced granuloma formation was abrogated if spleen cells from mice chronically infected with *S. mansoni* were pretreated with anti-Ly 1.1 alloantiserum and complement. Based on this finding and previous data (8) demonstrating a requirement for Ia^+ T cells in adoptive transfer of granuloma modulation, they proposed that the granulomatous inflammation is maintained by Ly 1^+, Ia^- cells, whereas the intensity of the response is regulated by Ly 1^+,Ia^+ and Ly 2^+,Ia^+ cells.

The work presented in this paper provides evidence to suggest that in addition to exerting suppressor regulatory activity over granuloma formation in *S. mansoni*-infected animals, T cells bearing the Ly 2 or I-J phenotype also may modulate the granulomatous inflammatory response during systemic infection of mice by *H. capsulatum*. However, proof of this awaits quantitative demonstration that adoptive transfer of Ly 2 and I-J antigen-bearing cells from *H. capsulatum*-infected mice to syngeneic or congenic recipients sensitized to antigens of *H. capsulatum* can regulate granuloma formation in the recipients.

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LITERATURE CITED

1. **Artz, R. P., and W. E. Bullock.** 1979. Immunoregulatory responses in experimental disseminated histoplasmosis: lymphoid organ histopathology and serological studies. Infect. Immun. **23**:884–892.
2. **Artz, R. P., and W. E. Bullock.** 1979. Immunoregulatory responses in experimental disseminated histoplasmosis: depression of T-cell-dependent and T-effector responses by activation of splenic suppressor cells. Infect. Immun. **23**:893–902.
3. **Cantor, H., and E. A. Boyse.** 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. I. The generation of functionally distinct T-cell subclasses is a differentiative process independent of antigen. J. Exp. Med. **141**:1376–1389.
4. **Cantor, H., and E. A. Boyse.** 1975. Functional subclasses of T lymphocytes bearing different Ly antigens. II. Cooperation between subclasses of Ly^+ cells in the generation of latter activity. J. Exp. Med. **141**:1390–1399.
5. **Cantor, H., and R. K. Gershon.** 1979. Immunological circuits: cellular composition. Fed. Proc. **38**:2058–2064.
6. **Cantor, H., and I. Weissman.** 1976. Development and function of subpopulations of thymocytes and T lymphocytes. Prog. Allergy **20**:1–64.
7. **Chensue, S. W., and D. L. Boros.** 1980. 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.
8. **Chensue, S. W., S. R. Wellhausen, and D. L. Boros.** 1981. Modulation of granulomatous hypersensitivity. II. Participation of $Ly1^+$ and $Ly2^+$ T lymphocytes in the suppression of granuloma formation and lymphokine production in *Schistosoma mansoni* infected mice. J. Immunol. **127**:363–367.
9. **Cunningham, A. J., and A. Szenberg.** 1968. Further improvements in the plaque forming technique for detecting simple antibody forming cells. Immunology **14**:599–600.
10. **Eardley, D. D., J. Hugenberger, L. McVay-Boudreau, F. W. Shen, R. K. Gershon, and H. Cantor.** 1978. Immunoregulatory circuits among T-cell sets. I. T helper cells induce other T cell sets to exert feedback inhibition. J. Exp. Med. **147**:1106–1115.
11. **Germaine, R. H., and B. Benacerraf.** 1980. Helper and suppressor T cell factors. Springer Semin. Immunopathol. **3**:93–127.
12. **Green, D. R., D. D. Eardley, A. Kimura, D. B. Murphy, K. Yamauchi, and R. K. Gershon.** 1981. Immunoregulatory circuits which modulate responsiveness to suppressor cell signals: characterization of an effector cell in

the contrasuppressor circuit. Eur. J. Immunol. **11**:973–980.

13. **Green, W. F., and D. G. Colley.** 1981. Modulation of *Schistosoma mansoni* egg-induced granuloma formation: I-J restriction of T cell mediated suppression in a chronic parasite infection. Proc. Natl. Acad. Sci. U.S.A. **78**:1152–1156.
14. **Julius, M. H., E. Simpson, and L. A. Herzenberg.** 1973. A rapid method for the isolation of functional thymus derived murine lymphocytes. Eur. J. Immunol. **3**:645–649.
15. **Ledbetter, J. A., and L. A. Herzenberg.** 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol. Rev. **47**:63–90.
16. **Marshak-Rothstein, A., P. Fink, T. Gridley, D. H. Raulet, M. J. Bevan, and M. L. Gefter.** 1979. Properties and applications of monoclonal antibodies directed against determinants of the Thy 1 locus. J. Immunol. **122**:2491–2497.
17. **Mishell, R. I., and R. W. Dutton.** 1967. Immunization of dissociated spleen cell cultures from normal mice. J. Exp. Med. **126**:423–442.
18. **Murphy, D. B.** 1978. The I-J subregion of the murine H-2 gene complex. Springer Semin. Immunopathol. **1**:111–131.
19. **Murphy, D. B., L. A. Herzenberg, K. Okumura, L. A. Herzenberg, and H. O. McDevitt.** 1976. A new I subregion (I-J) marked by a locus (Ia-4) controlling surface determinants on suppressor T lymphocytes. J. Exp. Med. **144**:699–712.
20. **Nickerson, D. A., R. A. Havens, and W. E. Bullock.** 1981. Immunoregulation in disseminated histoplasmosis: characterization of splenic suppressor cell populations. Cell. Immunol. **60**:287–297.
21. **Warr, G. W., J. C. Lee, and J. J. Marchalonis.** 1978. Null cells in the mouse possess membrane immunoglobulins. J. Immunol. **121**:1767–1772.