Brief Definitive Report

PARTICIPATION OF SUPPRESSOR T CELLS IN THE IMMUNOSUPPRESSIVE ACTIVITY OF A HETEROANTISERUM TO HUMAN Ia-LIKE ANTIGENS (p23,30)

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The genetic information for a number of distinct immune functions in mice can be assigned to one (or more) subregions of the *I*-region, which is located within the major histocompatibility complex (*H-2*) of chromosome 17 (1, 2). Cell surface antigens encoded by genes that map within the *I*-region of *H-2* are called Ia (immune response-associated) antigens (3, 4). Unlike determinants encoded within the *K* and *D* regions of *H-2*, Ia-antigens appear predominantly on B cells and monocytes. However, a subset of T cells may express Ia antigens. In particular, the *I-J* subregion contains the genetic information for surface-membrane markers found on suppressor T cells and a special class of helper T cells (2, 5).

In humans, the major histocompatibility genes map within the *HLA* complex of chromosome 6, and genes within the *HLA-D* region control the expression of antigens that appear to be the homologues of murine Ia antigens (6, 7). Sera from multiparous women and allosensitized individuals may contain antibodies reactive to B cells and monocytes, and such alloantibodies have been used to characterize human Ia-like antigens (8, 9). These antigens exist as a bimolecular glycoprotein complex (10), and they comprise the so-called *DR* system of *HLA* antigens. The *DR* determinants contain a nonpolymorphic antigenic component, which can be detected by heteroantisera raised in rabbits immunized against glycoproteins with an ~28,000–33,000 mol wt derived from B cells (11, 12).

Heteroantisera and alloantisera directed against human Ia-like antigens appear to interact with the same 28,000- to 33,000-dalton glycoprotein complex (13, 14). By analogy to murine systems, human Ia-like antigens are most readily evident on B cells and monocytes. However, some T cells (especially after exposure to activating stimuli) express Ia-like antigens (15–18). The role of Ia antigens in the regulation of human immune responses has, as yet, not been defined.

The purpose of this article is to report our findings that antibodies to nonpolymorphic Ia-like antigens (p23,30) can depress the production of immunoglobulins by normal human B cells under conditions of polyclonal activation, and that a maximal inhibitory effect requires the presence of a radiosensitive subset of regulatory T cells in our experimental system.

Materials and Methods

In Vitro Biosynthesis of Immunoglobulin. To study the transition of circulating normal human lymphocytes into immunoglobulin-secreting plasma cells, we cultured 2×10^6 unseparated cells

in the presence of pokeweed mitogen at 37°C in 5% CO_2 in RPMI-1640 media that contained 10% heat-inactivated fetal calf serum, 4 mM L-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin. The techniques used have been previously reported in detail (19). We measured the cumulative secretion of IgM, IgG, and IgA by heavy-chain specific double-antibody radioimmunoassays essentially as previously described for IgE (20). We prepared both T cells and B cells, freed of T cells, by a combination of immunoabsorbent-column purification and spontaneous rosette formation with sheep erythrocytes (21). Before use, sheep erythrocytes were removed from the T-cell population by exposure to ACK lysis buffer (NH_4Cl, $KHCO_3$, EDTA, and distilled H_2O). Pokeweed mitogen-stimulated B cells, rigorously freed of T cells, synthesize little or no immunoglobulins, nor do they show a substantial level of proliferation in vitro. The ability of added cells to restore immunoglobulin production by these purified B cells is an indication of helper cell activity (21). The capacity of cells to depress immunoglobulin production by indicator B cells in the presence of adequate helper cell function is a measure of suppressor cell activity (19). Normal circulating T-cell populations represent a heterogeneous mixture of potential helper and suppressor cell subsets.

Antiserum to Ia-like Antigens (p23,30). We prepared a rabbit antiserum to nonpolymorphic human Ia-like antigens. The papain-solubilized, purified p23,30 protein complex was the immunogen as previously described (11). The antiserum used selectively causes the bimolecular immunoprecipitation profile of membrane proteins characteristic of Ia-like antigens using sodium dodecyl sulfate polyacrylamide gel analysis. The serologic and biochemical properties of the antiserum used in the experiments described in this article are reported in detail elsewhere (7, 14). To test the effect of this antiserum upon in vitro immunoglobulin production, 1 μl was added to various populations of cells at the start of the culture (volume = 1 ml) and left in for the entire 12-d culture period. Normal rabbit serum served as the control. Both the antiserum and the normal serum reagents were heat-inactivated (56°C for 30 min) before use. Complement was not added.

Results

Table I illustrates the effect of antiserum to human Ia-like antigens on the production of immunoglobulin by normal unseparated lymphocytes. The antiserum decreased pokeweed mitogen-driven immunoglobulin production in vitro using unseparated lymphocytes from three unrelated normal individuals. The level of suppression ranged from ~65 to 95%. Normal rabbit serum did not inhibit immunoglobulin production. Because normal B cells and monocytes readily express Ia-like surface membrane determinants, this effect is open to a number of interpretations, including a direct antibody-induced inhibition of the B cells within the unseparated lymphocyte target population. Moreover, it is difficult to standardize experiments with unseparated lymphocyte populations because the regulatory T-cell:indicator B-cell ratios may vary. For these reasons, we next concentrated on experiments involving purified

TABLE I

*Inhibitory Effect of a Heteroantiserum to Human Ia-like Antigens upon Immunoglobulin Production by Normal Lymphocytes In Vitro**

Rabbit serum reagent added	IgM	IgG	IgA
		ng secreted	
α-Ia	580	258	328
NRS	2,760	3,836	976

* 2×10^6 normal mononuclear cells (prepared by Ficoll-Hypaque gradient density isolation) were cultured for 12 d. A rabbit antiserum to p23,30 (α-Ia) or normal rabbit serum (1 μl/final 1 ml culture volume) was added at the beginning and left in throughout the culture period. Pokeweed mitogen was the polyclonal activator. Complement was not added. NRS, normal rabbit serum.

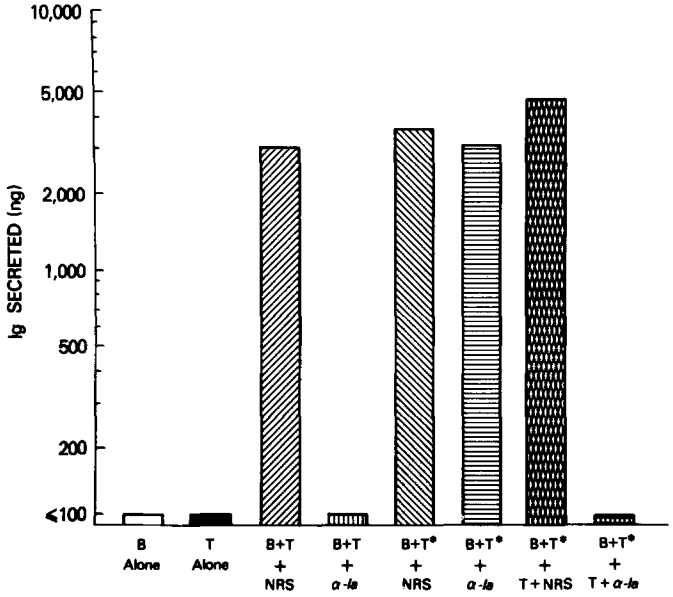

FIG. 1. Immunosuppressive effect of a heteroantiserum to human Ia-like antigens requires the presence of a radiosensitive T-cell subset. Here 5×10^5 normal B cells were cultured in the presence of pokeweed mitogen without T cells, or with various combinations of irradiated or unirradiated autologous T cells. In this system, irradiation was used to neutralize the suppressor pool of normal T cells without appreciably impairing the helper pool. 5×10^5 unirradiated and/or 5×10^5 irradiated T cells were added to the indicator B cells. The antiserum (without complement) brought about a suppressor effect, but only when unirradiated T cells were also added to the system. Ig values represent IgA, but IgM and IgG results were comparable. *Cells received 2,000 R x irradiation in advance. *α-Ia*, rabbit antiserum to p23,30; NRS, normal rabbit serum.

T cells and B cells to help us resolve whether the functional capacity of T cells influenced the ability of the antiserum to suppress in vitro humoral immune function. Five separate experiments were performed with purified B/T populations from four unrelated normal individuals.

In these experiments, we took advantage of the relative radioresistance of normal helper T cells and the relative radiosensitivity of normal suppressor T cells. If the antiserum to Ia-like antigens acted directly at the level of B cells or monocytes, suppression of immunoglobulin synthesis would be expected when we cultured B cells with either irradiated or unirradiated autologous T cells. On the other hand, if the antiserum to Ia-like antigens had to function through a regulatory T-cell intermediary for the expression of its inhibitory activity, one would expect depressed immunoglobulin synthesis only when unirradiated T cells were present. The results shown in Fig. 1 provide evidence that the inhibitory effect exerted by the antiserum to Ia-like antigens occurred only when unirradiated T cells were provided. The data indicate that normal B cells did not produce immunoglobulin alone. When irradiated or unirradiated autologous T cells were provided as the source of helper activity, there was a restoration of immunoglobulin production. The addition of the antiserum to Ia-like antigens (p23,30) to the mixture of B cells and unirradiated T cells profoundly depressed immunoglobulin production. Suppression did not take place when the indicator population was a mixture of B cells and irradiated T cells exposed to this

antiserum. This observation suggests that a radiosensitive subset of autologous T cells is necessary for the anti-Ia suppressor effect in this system. The suppressor effect was restored when unirradiated autologous T cells were once again introduced into the system. In the five experiments performed with purified B cells, inhibition of immunoglobulin production in the presence of unirradiated autologous T cells ranged from 88 to 99% when the antiserum to Ia-like antigens was added to the cultures. The levels of suppression for IgM, IgG, and IgA were comparable.

When we used unirradiated T cells and indicator B-cell populations from unrelated normal individuals, the antiserum to Ia-like antigens produced only a modest or no suppressor effect. This suggests that there may be a genetic preference for the suppressor T-cell effect brought about by the presence of antibodies to Ia-like determinants in this system.

Discussion

In analyzing the participation of suppressor cells in the inhibitory effect brought about by antibodies to human Ia-like antigens in these kinds of experiments, it is necessary to obtain a starting population of indicator B cells rigorously depleted of T cells. Operationally, the indicator B-cell population should not secrete substantial quantities of immunoglobulins when cultured alone in the presence of pokeweed mitogen. When the indicator B-cell population alone secretes substantial quantities of immunoglobulins, one must assume that there is a contaminating pool of regulatory T cells. When such contaminating T cells are present, it may be impossible to distinquish between the influence of regulatory T cells and a direct inhibitory action of the antibodies on B cells or monocytes.

The data presented in this report provide evidence that human Ia-like antigens can play a role in the T-cell regulatory control of humoral immune function. An antiserum to purified Ia-like components (p23,30) suppressed polyclonally activated immunoglobulin production in vitro. This form of suppression was most clearly evident when various combinations of normal B cells and autologous irradiated or unirradiated normal T cells were used as the indicator system. The suppressor effect does not appear to operate through a simple action at the level of target B cells or monocytes alone.

In a wide range of experimental systems, antibodies to Ia antigens can depress both cellular and humoral immune reactions (22–27). Most workers have concluded that anti-Ia antibodies exert their inhibitory effects by directly interfering with B-cell or monocyte functions. There are experimental systems in which it appears unnecessary to postulate more complex phenomena, such as an alteration of regulatory T-cell function, to explain the activity of antibodies to Ia antigens. However, our data would suggest that in certain systems the suppressive effects of such antibodies cannot be adequately explained without considering the role of regulatory T cells.

Recent experiments by Fu et al. (28) indicate that human T cells involved in the generation of allogeneic helper activity express Ia-like antigens. Therefore, one should keep in mind that under some experimental conditions antibodies to Ia could impair certain helper cell functions.

In the experimental system reported in this article, the antibodies to Ia-like antigens did not appreciably affect helper activity (Fig. 1, B cells + irradiated T cells), but rather the antibodies induced the emergence of suppressor T-cell activity. From our

data, we infer that at whatever point (B cell, monocyte, or T cell) anti-Ia antibodies perturb the immune system initially, the final suppressor effect may require the participation of suppressor T cells. This has both theoretical and clinical implications.

Summary

We studied the effects of an antiserum to human Ia-like antigens (p23,30) upon the polyclonal activation of normal B cells (cultured with various combinations of irradiated and unirradiated T cells) to become immunoglobulin-secreting cells after stimulation with pokeweed mitogen in vitro. We found that the antiserum suppressed immunoglobulin production. The inhibitory effect did not appear to result from a simple interaction at the B-cell/monocyte level alone. Rather, the inhibitory effect required the presence of a radiosensitive subset of autologous suppressor T cells.

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References

1. Shreffler, D. C., and C. S. David. 1975. The *H-2* major histocompatibility complex and the *I* immune response region: genetic variation, function, and organization. *Adv. Immunol.* **20:** 125.
2. Benacerraf, B., and R. N. Germain. 1978. The immune response genes of the major histocompatibility complex. *Immunol. Rev.* **38:**70.
3. McDevitt, H. O., T. L. Delovitch, J. L. Press, and D. B. Murphy. 1976. Genetic and functional analysis of the Ia antigens: their possible role in regulating the immune response. *Transplant. Rev.* **30:**197.
4. Sachs, D. H. 1976. The Ia antigens. *Contemp. Top. Mol. Immunol.* **5:**1.
5. Tada, T., T. Takemori, K. Okumura, M. Nonaka, and T. Tokuhisa. 1978. Two distinct types of helper T cells involved in the secondary antibody response: independent and synergistic effects of Ia^- and Ia^+ helper T cells. *J. Exp. Med.* **147:**446.
6. Van Rood, J. J., A. van Leeuwen, A. Termijtelen, and J. J. Keuning. 1976. B-cell antibodies, Ia-like determinants, and their relation to MLC determinants in man. *Transplant. Rev.* **30:**122.
7. Lunney, J. K., D. L. Mann, and D. H. Sachs. 1979. Sharing of Ia antigens between species. III. Ia specificities shared between mice and human beings. *Scand. J. Immunol.* **10:**403.
8. Winchester, R. J., S. M. Fu, P. Wernet, H. G. Kunkel, B. Dupont, and C. Jersild. 1975. Recognition by pregnancy serums of non-*HLA* alloantigens selectively expressed on B lymphocytes. *J. Exp. Med.* **141:**924.
9. Albrechtsen, D., B. G. Solheim, and E. Thorsby. 1977. Serological identification of five *HLA-D* associated (Ia-like) determinants. *Tissue Antigens.* **9:**153.
10. Springer, T. A., J. F. Kaufman, C. Terhorst, and J. L. Strominger. 1977. Purification and structural characterization of human *HLA*-linked B-cell antigens. *Nature (Lond.).* **268:**213.
11. Humphreys, R. E., J. M. McCune, L. Chess, H. C. Herrman, D. J. Malenka, D. L. Mann, P. Parham, S. F. Schlossman, and J. L. Strominger. 1976. Isolation and immunologic characterization of a human B-lymphocyte-specific, cell surface antigen. *J. Exp. Med.* **144:** 98.
12. Snary, D., C. J. Barnstable, W. F. Bodmer, P. N. Goodfellow, and M. J. Crumpton. 1977. Cellular distribution, purification, and molecular nature of human Ia antigens. *Scand. J. Immunol.* **6:**439.
13. Giphart, M. J., J. F. Kaufman, A. Fuks, D. Albrechtsen, B. G. Solheim, J. W. Bruning, and J. L. Strominger. 1977. *HLA-D* associated alloantisera react with molecules similar to Ia antigens. *Proc. Natl. Acad. Sci. U. S. A.* **74:**3533.

14. Mann, D. L., J. Kaufman, H. Orr, R. Robb, and J. Strominger. 1979. Serologic and structural studies of DR-related antigens. *Transplant. Proc.* **11**:668.
15. Evans, R. L., T. J. Faldetta, R. E. Humphreys, D. M. Pratt, E. J. Yunis, and S. F. Schlossman. 1978. Peripheral human T cells sensitized in mixed leukocyte culture synthesize and express Ia-like antigens. *J. Exp. Med.* **148**:1440.
16. Metzgar, R. S., J. Bertoglio, J. K. Anderson, G. D. Bonnard, and F. W. Ruscetti. 1979. Detection of *HLA-Drw* (Ia-like) antigens on human T lymphocytes grown in tissue culture. *J. Immunol.* **122**:949.
17. Ko, H.-S., S. M. Fu, R. J. Winchester, D. T. Y. Yu, and H. G. Kunkel. 1979. Ia determinants on stimulated human T lymphocytes. Occurrence on mitogen- and antigen-activated T cells. *J. Exp. Med.* **150**:246.
18. Greaves, M. F., W. Verbi, H. Festenstein, C. Papasteriadis, D. Jaraquemada, and A. Hayward. 1979. "Ia-like" antigens on human T cells. *Eur. J. Immunol.* **9**:356.
19. Waldmann, T. A., S. Broder, M. Durm, M. Blackman, R. M. Blaese, and W. Strober. 1974. Role of suppressor T cells in pathogenesis of common variable hypogammaglobulinemia. *Lancet.* **II**:609.
20. Waldmann, T. A., S. H. Polmar, S. T. Balestra, M. C. Jost, R. M. Bruce, and W. D. Terry. 1972. Immunoglobulin E in immunologic deficiency diseases. II. Serum IgE concentration of patients with acquired hypogammaglobulinemia, myotonic dystrophy, intestinal lymphangiectasia, and Wiskott-Aldrich syndrome. *J. Immunol.* **109**:304.
21. Broder, S., R. L. Edelson, M. A. Lutzner, D. L. Nelson, R. P. MacDermott, M. E. Durm, C. K. Goldman, B. D. Meade, and T. A. Waldmann. 1976. The Sézary syndrome. A malignant proliferation of helper T cells. *J. Clin. Invest.* **58**:1297.
22. Bluestein, H. G. 1974. Alloantiserum-mediated suppression of histocompatibility-linked *Ir*-gene-controlled immune responses. Suppressive effects of IgG fragments derived from alloantisera. *J. Exp. Med.* **140**:481.
23. Marrack, P., and J. W. Kappler. 1977. Anti-Ia inhibits the activity of B cells but not T cell-derived helper mediator. *Immunogenetics.* **4**:541.
24. Thomas, D. W., U. Yamashita, and E. M. Shevach. 1977. Role of Ia antigens in T cell activation. *Immunol. Rev.* **35**:97.
25. Niederhuber, J. E. 1978. The role of I region gene products in macrophage-T lymphocyte interaction. *Immunol. Rev.* **40**:28.
26. Schwartz, R. H., C. S. David, M. E. Dorf, B. Benacerraf, and W. E. Paul. 1978. Inhibition of dual *Ir* gene-controlled T-lymphocyte proliferative response to poly($Glu^{56}Lys^{35}Phe^{9}$)$_n$ with anti-Ia antisera directed against products of either *I-A* or *I-C* subregion. *Proc. Natl. Acad. Sci. U. S. A.* **75**:2387.
27. Friedman, S. M., J. M. Breard, R. E. Humphreys, J. L. Strominger, S. F. Schlossman, and L. Chess. 1977. Inhibition of proliferative and plaque-forming cell responses by human bone-marrow-derived lymphocytes from peripheral blood by antisera to the p23,30 antigen. *Proc. Natl. Acad. Sci. U. S. A.* **74**:711.
28. Fu, S. M., N. Chiorazzi, C. Y. Wang, G. Montazeri, H. G. Kunkel, H. S. Ko, and A. B. Gottlieb. 1978. Ia-bearing T lymphocytes in man. Their identification and role in the generation of allogeneic helper activity. *J. Exp. Med.* **148**:1423.