Brief Definitive Report

ASSOCIATION BETWEEN Ia ANTIGENS AND THE Fc RECEPTORS OF CERTAIN T LYMPHOCYTES

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Ia (*Ir* associated) antigens and Fc receptors have previously been shown to be associated on the murine B-lymphocyte surface (1). Pretreatment of lymphocytes with anti-Ia antibodies, as well as Fab fragments thereof, specifically inhibited the binding of complexed Ig to B lymphocytes. In contrast, antibodies against the *K* and *D* regions of the *H-2* complex did not produce such inhibition. This observation has been confirmed in the mouse (2, 3), and similar observations have been made in both man (4) and the rat (5). One laboratory was unable to demonstrate a specific association (6). Recent experiments (7–9) have shown that Ia antigens and Fc receptors on B lymphocytes are probably not identical, but rather appear to be closely associated with each other on the B-lymphocyte membrane. This conclusion is based on the observation that capping of Fc receptors does not appear to alter the distribution or detectability of most Ia antigens (7–9) and certain lymphoid cell lines have been identified which bear "Ia-like" antigens but not detectable Fc receptors (10).

Although more difficult to detect, both Fc receptors (11–13) and Ia antigens (14–17) appear to be present on at least a subpopulation of T lymphocytes in both the thymus and spleen of mice. The experiments reported here provide evidence that Ia antigens are also specifically associated with the Fc receptors of some of these T lymphocytes.

Materials and Methods

6- to 8-wk-old male mice were utilized. Strains C57BL/10Sn (B10), B10.A/SgSn, B10.BR/SgSn, and B10.A(2R) were purchased from The Jackson Laboratory, Bar Harbor, Maine. Strain A.TL was obtained from our own breeding colony established from stock kindly provided by Doctors D. C. Shreffler and C. S. David, Washington University School of Medicine, St. Louis, Mo.

Alloantibodies were prepared as previously described (18, 19) and the specifications utilized are listed in Table I. All antisera were decomplemented, ultracentrifuged to remove complexes, and used either undiluted or at dilutions which were on the plateau of cytotoxic killing for the strain of cells being tested, as previously described (1). $F(ab')_2$ fragments were prepared by a modification[1] of the method of Nisonoff et al. (20). This $F(ab')_2$ preparation was free of intact antibody and Fc fragments by the criteria of size, loss of complement-mediated cytotoxic activity, and failure to react with specific anti-γG1Fc and anti-γG2Fc reagents (kindly provided by Dr. R. Asofsky, NIH, Bethesda, Md.).

Preparation of single cell suspensions from spleen and thymus, and detection by fluorescence of surface Ig using fluorescein isothiocyanate-conjugated rabbit antimouse Ig were performed as

[1] Cone, J. L., J. E. Ricks, and D. H. Sachs. Manuscript in preparation.

TABLE I

Inhibition of Binding of Antigen-Antibody Complexes to Murine Thymocytes by Anti-Ia Antibodies

Exp.	Strain	Cells binding complexes	Serum	Specificity	Inhibition
		%			%
1	B10.A	21.0	Normal A/J	–	−7
			A.TH anti-A.TL	*I* region	62
2	B10	10.0	Normal B10.A	–	10
			B10 anti-B10.BR	–	5
			B10.A anti-B10	Whole *H-2* complex	65
			B10.A × A/J anti-B10.D2	*I-A* subregion	65
3	B10.A	18.0	B10.A anti-B10	–	3
			B10.A(4R) anti-B10.A(2R)	*I-C* subregion	67
4	B10.BR	10.0	B10.BR anti-B10	–	−5
			A.TH anti-A.TL	*I* region	65
			B10.A(4R) anti-B10.A(2R)	*I-C* subregion	45
5	B10	18.0	Normal B10.A	–	−8
			B10 anti-B10.BR	–	3
			B10.BR anti-B10	Whole *H-2* complex	69
			B10.BR anti-B10 $F(ab')_2$	Whole *H-2* complex	64
6	A.TL	19.5	Normal A/J	–	−5
			A.TH anti-A.TL	*I* region	74
			A/J anti-A.SW	*K* region	3
7	B10.A(2R)	13.5	Normal B10.A	–	4
			B10 anti-B10.A	*H-2* complex except *D* region	67
			B10.A × A/J anti-B10	*D* region	−4
8	B10.A	17.5	A.TH anti-A.TL	*I* region	49
			B10.A(4R) anti-B10.A(2R)	*I-C* subregion	46
			B10 × AQR anti-B10.A	*K* region	6
			B10.BR anti-B10	*D* region public specificities	3
9	B10	14.0	B10 anti-B10.BR	–	−7
			B10.BR anti-B10	Whole H-2 complex	57
			B10.A × A/J anti-B10.D2	*I-A* subregion	50
			AKR anti-C3H	Thy-1.2	−7

previously described (1). Thy 1.2 antigen was detected by indirect immunofluorescence using AKR anti-C3H antiserum and fluorescein isothiocyanate-conjugated goat antimouse γG2 (kindly provided by Dr. R. Asofsky). The latter antiserum was raised against the Fc fragment of a γG2 myeloma (MPC 37) and was purified by affinity chromatography on a γG2 column. This reagent used alone stained 1–2% of normal spleen lymphocytes.

The method for detection by indirect fluorescence of T-cell Fc receptors using soluble antigen-antibody complexes is the subject of a separate publication (21). Antigen [TNP_{16} bovine serum albumin (BSA) or TNP_{30}BSA] was prepared by dissolving BSA (100 mg, 1.47 μmol) in 10 ml of 0.1 M borate buffer, pH 9.0, and adding trinitrobenzene sulfonate (TNBS) in phosphate-buffered saline (PBS), pH 7.2 (respectively, 2.35 ml of 10 mM TNBS-23.5 μmol, or 1.0 ml of 100 mM TNBS-100 μmol). The solution was mixed well, incubated at 37°C for 1 h, and then dialyzed overnight against PBS, pH 7.2. The degree of conjugation achieved was determined by spectroscopy. Affinity purified rabbit IgG anti-TNP was prepared by hyperimmunizing rabbits with heavily substituted TNP-keyhole limpet hemocyanin (KLH) prepared similarly to TNP-BSA. The course of immunization and purification of antibody were followed by anti-TNP hemagglutination titers. The Ig was isolated from the antiserum by precipitation with $(NH_4)_2SO_4$ and then affinity purified on DNP-lysine-coupled Sepharose. The specific antibody was eluted from the column with 0.1 M acetic acid and immediately neutralized with Tris. The monomeric IgG was separated from

IgM and aggregated IgG by gel filtration on Sephadex G200. The IgG fraction was concentrated (generally to 1 mg/ml) by vacuum dialysis, dialyzed against PBS, pH 7.2, aliquoted, and stored at −20°C. $F(ab')_2$ fragments were prepared by pepsin digestion and chromatography on Sephadex G200 (20). The $F(ab')_2$ preparations had a hemagglutination titer very similar to that of the intact antibody, but were unable to lyse TNP-red blood cells in the presence of complement, and did not react with antirabbit Fc antibodies in Ouchterlony double diffusion. A precipitin curve was used to establish the equivalence point for each batch of antigen and antibody. A fixed amount of antibody (100 μl of 1 mg/ml) was mixed with varying amounts of antigen (0.3–30 μg) in a fixed volume (200 μl). The mixture was incubated for 1 h at 37°C and then overnight at 4°C (it should be noted that the complexes prepared with $TNP_{16}BSA$ remained soluble unless incubated in the cold). After washing, the protein precipitates were quantitated by Lowry determination. A typical equivalence point was 100 μg antibody to 3 μg antigen. Some variation was noted depending on the degree of conjugation of the antigen and the particular batch of antibody. Complexes were prepared fresh daily by incubating antigen and antibody at 23°C for 1 h at ratios ($TNP_{30}BSA$, fourfold antigen excess; $TNP_{16}BSA$, fourfold antigen excess or "equivalence") which gave soluble complexes which were easily detectable. The preformed complexes (100 μl of various concentration, see Results) were mixed with the lymphocyte suspension (25 μl containing 5×10^5 cells) and incubated for 30 min at 23°C. The lymphocytes were washed thoroughly and stained with fluorescein isothiocyanate or tetramethylrhodamine isothiocyanate-conjugated goat antirabbit IgG (Cappel Laboratories, Inc., Downingtown, Pa.; lots 7853 and 7707) which had been absorbed with Sepharose-coupled normal mouse ascites proteins or IgG. The lymphocytes were again washed thoroughly, wet mounted, and evaluated by fluorescence and phase microscopy (1) for the percentage of positive cells. Inhibition studies were performed by incubating the lymphocytes in 100 μl of antiserum for 30 min at 4°C, washing thoroughly, and then assaying for Fc receptors as described above.

Results

Detection by Indirect Fluorescence of the Binding of Soluble Antigen-Antibody Complexes to T Lymphocytes. The percentage of lymphocytes which bound soluble antigen-antibody complexes as detected by indirect fluorescence was dependent on the concentration of complexes used, with two plateaus being observed. At concentrations between 6 and 50 μg/ml, <0.5% of thymic lymphocytes and 40–55% of splenic lymphocytes were positive. The latter cells were essentially overlapping (~90%) with the surface Ig-bearing population as determined by double-labeling experiments, and were therefore primarily B lymphocytes. At concentrations of complexes >400 μg/ml 10–20% of thymocytes and 65–80% of splenic lymphocytes were positive. Since the thymocytes were <0.5% surface Ig positive and 99.0% theta positive, essentially all the cells binding complexes in the thymus are T cells. Double-labeling experiments with Thy-1 and complexes on splenocytes indicated that 40–50% of the Thy-1-positive cells in the spleen bound complexed Ig. Control experiments showed that antibody alone or complexes made with IgG $F(ab')_2$ anti-TNP did not bind, indicating that the assay was specific and that binding appeared to require the Fc portion of the Ig molecule. These results are in general agreement with those of other laboratories (11–13).

Inhibition of Binding of Antigen-Antibody Complexes to T Lymphocytes by Anti-Ia Antibodies. Thymocytes pretreated with antisera containing antibodies against antigens determined by the *I* region of the *H-2* complexes were inhibited (45–74%) in their ability to bind antigen-antibody complexes (Table I). Lack of complete inhibition did not appear to be due to insufficient antibody since the antisera were often used at concentrations in vast excess (>100-fold) of that needed to maximally inhibit binding to the Fc receptors of B lymphocytes.

Antisera specific for the *I-A* and *I-C* subregions also produced inhibition. Although the degree of inhibition was sometimes less than that obtained with antisera which reacted with antigens determined by the entire *I* region (exps. 4 and 9), this was not a consistent observation (exps. 2 and 8). Control experiments indicated that: (*a*) normal sera and immune sera which did not react with the cells did not produce inhibition; (*b*) antibodies which bound to other antigens on the cell surface (Thy-1.2 and antigens determined by the *K* and *D* regions of the *H-2* complex) did not cause inhibition (exps. 6–9); and (*c*) $F(ab')_2$ fragments inhibited as well as the antibodies from which they were prepared (exp 5).

Discussion

The present studies have shown that antibodies against Ia antigens inhibit the binding of antigen-antibody complexes to the Fc receptors of some T lymphocytes. This inhibition was not due to the Fc portion of the antibody molecule since $F(ab')_2$ fragments also inhibited and was specific in that antibodies against Thy-1.2 antigen, as well as antigens determined by the *K* and *D* regions of the *H-2* complex did not inhibit. These results provide evidence that Fc receptors and Ia antigens are associated on at least some T cells and that the populations of T lymphocytes which bear Ia antigens (14–17) and Fc receptors (11–13) are at least partially overlapping. Similar results for T cells in the spleen have been obtained by Stout and associates.[2]

It is unclear why only partial inhibition of binding of Ig complexes to T-cell Fc receptors by anti-Ia antibodies was obtained. One possibility is that anti-Ia antibodies inhibit the Fc receptors of all T cells which bear them equally, but by statistical chance a certain fraction of the cells do not have their staining reduced to below visual threshold. Alternatively, there may be two distinct populations of T lymphocytes bearing Fc receptors: one population whose receptors are associated with Ia antigens and another whose receptors are not. We favor the latter interpretation because (*a*) the population of T cells still positive for Fc receptors were undiminished in the intensity of their staining (subjective microscopic evaluation), and (*b*) inhibition of cells in the spleen binding aggregated Ig was also partial (1), whereas purified surface Ig-positive cells were inhibited completely.[3] Further studies are needed to clarify this point.

While the Fc receptors of B cells and some T cells appear to be associated with Ia antigens, this is not true for all immunorelevant cells bearing Fc receptors. Anti-Ia antisera do not inhibit the binding of Ig complexes to the Fc receptors of macrophages (reference 3, 22) or K cells.[3] The specific association of Fc receptors with antigens determined by the *I* region of the *H-2* complex as well as the restriction of this association to certain cell types suggests that the relationship between Ia antigens and Fc receptors may be important in the regulation of the immune response.

Summary

The Fc receptors of thymic and splenic T lymphocytes were detected using indirect immunofluorescence and soluble antigen-antibody complexes. 10–20%

[2] Stout, R. D., D. Murphy, H. O. McDevitt, and L. A. Herzenberg. Manuscript in preparation.

[3] Dickler, H. B., D. L. Nelson, and D. H. Sachs. Manuscript in preparation.

of thymocytes and 40–50% of Thy-1-positive splenic lymphocytes bound antigen-complexed Ig. The binding to thymocytes was partially inhibited (45–74%) by antibodies against antigens determined by the *I* region of the *H-2* complex, but not by antibodies against *K*- or *D*-region antigens or Thy-1 antigen. The inhibition did not require the Fc portion of the inhibiting antibody. These results provide evidence that Ia antigens and the Fc receptors of some T lymphocytes are associated, and that the populations of T cells which bear these moieties at least partially overlap.

The authors wish to thank J. L. Cone and M. T. Kubicek for excellent technical assistance.

Received for publication 4 March 1976.

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