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Ia ANTIGENS ON FcR POSITIVE T LYMPHOCYTES

Bob Stout, Donal Murphy, Hugh McDevitt and Leonard Herzenberg
Brandeis University and Stanford University

Treatment of splenic T lymphocytes with anti-Ia antiserum inhibits the binding of antigen-antibody complexes (AgAb) to the majority (>50%) of FcR$^+$ T lymphocytes. Utilizing the ability anti-Ia antisera to inhibit binding of AgAb as an assay for the presence of I-region controlled determinants, we were able to demonstrate that determinants controlled by loci mapping in the I-A and I-C, S, or G regions are present on FcR$^+$ T cells.

I. INTRODUCTION

For the past several years, we have been using flouresceinated antigen-antibody complexes (F*AgAb) to distinguish two distinct subpopulations of peripheral T cells - Fc receptor positive (FcR$^+$) and Fc receptor negative (FcR$^-$) T cells (1). Functional studies on populations of FcR$^-$ and FcR$^+$ T cells, purified on the fluorescence activated cell sorter (FACS), revealed that the FcR$^-$ T cell subpopulation a) responded poorly, if at all, to concanavalin A (con A) in the absence of FcR$^+$ T cells (2), b) contained the precursors of cytotoxic effectors of cell mediated lympholytic (CML) responses (3), and c) contained the helper T cells (1) but not the CML amplifier T cells (3). In contrast, the FcR$^+$ T cell subpopulation a) was responsive to con A (2), b) contained the differentiated cytotoxic effector cells of CML responses (3), and c) contained the CML amplifier T cells (3) but not the helper T cells (1). Both FcR$^-$ and FcR$^+$ T cells were capable of proliferating in mixed lymphocyte cultures upon exposure to allogeneic lymphocytes (3). Despite these functional distinctions between T cells bearing or lacking a detectable Fc receptor, the functional significance of the Fc receptor remained unclear.

An association of the Fc receptor on murine B lymphocytes and alloantigens controlled by loci mapping in the I-region of the H-2 complex (I-region associated or Ia antigens) has been reported (4,5). This association was based on the observation that anti-Ia antibodies specifically inhibit the binding of aggregated immunoglobulin or antigen-antibody complexes to the Fc receptors of B cells. Recently, Ia anti-

gens have been detected on at least a subpopulation of T cells (6-10). To determine whether the Ia antigens are expressed on the FcR^+ T cells, we examined the ability of specific anti-Ia antisera to inhibit the binding of AgAb to the Fc receptor of splenic T lymphocytes.

II. MATERIALS AND METHODS

Methods for preparation of complexes of egg albumin and anti-egg albumin, cell suspensions, and antisera, and for inhibition assays, fluorescence labeling, and fluorescence analysis have been described in previous publications (1,10). Male and female mice of the inbred strains B10, B10.HTT, B10.A (3R), B10.A (4R), B10.A (5R), A.TH, A.TL, and B10.BR were obtained from the colonies at Stanford University Department of Medicine.

III. RESULTS AND DISCUSSION

The proportion of splenic T cells labeled by a 30 minute incubation with fluoresceinated AgAb can be reduced 50-70% by treating the cells, prior to exposure to F*AgAb, with antisera directed against determinants controlled by the I-region of the H-2 complex (Table 1). This inhibition was serologically specific insofar as FcR^+ T cells from A.TL and B10.BR mice (I^k) were inhibited by A.TH anti-A.TL antiserum (anti-I^k) but not by A.TL anti-A.TH antiserum (anti-I^s). Conversely, the FcR^+ T cells from A.TH mice were inhibited by anti-I^s but not by anti-I^k (Table 1). The inhibitory effect of these antisera could not be ascribed to antibody against determinants controlled by loci mapping in the Tla-region since a) treatment of T cells with antiserum prepared against Tla-region determinants failed to inhibit subsequent binding of F*AgAb to the T cells, and b) T cells from B10.BR mice, which strain appears to have the same Tla-region as A.TH, were not inhibited by the A.TL anti-A.TH antiserum (Table 1).

Treatment of the T cells with either a T cell specific alloantiserum (anti-Thy 1.2) or xenoantiserum (rabbit anti-mouse brain) did not inhibit subsequent binding of F*AgAb (Table 1). Thus, the inhibition of binding of F*AgAb observed with anti-Ia sera does not appear to be a general effect of antibody reacting with T cell membrane determinants. Several mechanisms have been proposed to explain the inhibition of B cell FcR by anti-Ia antibodies (4,5,11). These hypothetical mechanisms differ in regard to whether or not there is a physical association between the Fc receptor and Ia antigens. The arguments for or against such an associa-

TABLE 1
Inhibition of AgAb Binding to T Lymphocytes by Anti-Ia Antisera

Mouse Strain	% FcR^+	% Decrease in Proportion FcR^+ T Cells[1] By: [2]Anti-Thy 1.2	[3]Anti-Tla	[4]Anti-I^s	[5]Anti-I^k
A.TL	26	-	0	4	54
A.TH	31	-	0	60	5
B10.BR	29	0	-	5	52

[1]Nylon wool purified T cells (≥ 95% Thy 1.2^+ as determined by cytotoxicity assay) were incubated 30 min. at 37°C with the designated antisera prior to labeling with AgAb (10). Fraction of FcR^+T cells and degree of inhibition was based on analysis of 10,000 viable cells.

[2]AKR anti-C3H Thy 1, used at 1:10, has 100% cytotoxicity endpoint of >1:50, for thymocytes under conditions used for FcR inhibition. Similar results were obtained with a heterologous rabbit anti-mouse brain serum.

[3]Anti-Tla 1,2,3; used at 1:10 dilution, has cytotoxicity titer of >1:2,000 for thymocytes. This sera was kindly provided by Dr. E. A. Boyse.

[4]A.TL anti-A.TH, used at 1:20 dilution.

[5]A.TH anti-A.TL, used at 1:20 dilution.

tion have been amply discussed elsewhere (4,5,11) and will not be elaborated here. One point of agreement is that in order for an antibody to interfere with the FcR on the cell membrane, that antibody must first bind to its antigen (5, 11). Therefore, the ability of anti-Ia antibodies to block the FcR on T cells demonstrates the existence of Ia antigens on peripheral FcR^+ T cells.

Serological studies with peripheral T lymphocytes (7,12, 13), con A stimulated blasts (9), and thymocytes (6,8,) suggested that at least some T lymphocytes express I-region determinants. Having established the presence of I-region determinants on FcR^+ T cells, experiments were undertaken to map the loci controlling these determinants. The data obtained from studies on the FcR inhibitory activity of anti-Ia

sera on splenic T cells from B10.HTT mice demonstrated that at least two loci control determinants present on FcR^+ T cells. The crossover in B10.HTT occurred between the I-J and I-E subregions, such that it carries the I-A, I-B, and I-J subregions derived from the $H\text{-}2^s$ haplotype and the I-E and I-C subregions derived from the $H\text{-}2^k$ haplotype. Treatment of B10.HTT splenic T cells with A.TL anti-A.TH (anti-I^s) antiserum resulted in a 50% reduction in the proportion of FcR^+ T cells labeled by F*AgAb, where as treatment with A.TH anti-A.TL (anti-I^k) antiserum resulted in a 30% reduction in FcR^+ T cells labeled (Table 2). It thus appears that loci mapping on opposite sides of the crossover in B10.HTT control determinants found on FcR^+ T cells.

The FcR inhibition data obtained with strains B10.A(4R) and B10 demonstrate that one of the loci discussed above maps in the I-A subregion. These two strains differ in the H-2K and I-A subregions. Serum produced by immunizing B10.A(4R) recipients with B10 donor tissue contains antibody against determinants controlled by both regions. Following absorption with EL-4 tumor cells to remove H-2K antibody, this serum inhibited binding of F*AgAb complexes to B10 FcR^+ T cells (Table 2). Similar results were obtained with a (B10.HTT x A.TFR5)F_1 anti-A.TL serum, which contains antibody reactive with I-A determinants carried by strain B10.A(4R) (Table 2).

Determinants controlled by the I-J subregion do not appear to be present on FcR^+ T cells, since a B10.A(3R) anti-B10.A(5R) serum, which was known to contain antibody against determinants controlled by the I-J subregion of the 5R haplotype, failed to inhibit the binding of F*AgAb complexes to B10.A(5R) FcR^+ T cells (Table 2). This conclusion was supported by the failure of (B10.HTT x A.TFR5)F_1 anti-A TL serum to inhibit binding of F*AgAb complexes to B10.A(5R) FcR^+ T cells.

A second locus controlling determinants on FcR^+ T cells appears to map to the right of the I-E subregion. Determinants controlled by the I-E subregion were initially detected by the cytotoxic reactivity of a (C3H.Q x B10.D2)F_1 anti-B10.AQR serum with B10.A(5R) spleen cell targets (14-15). A serum produced in a similar strain combination ((B10.T(6R) x B10.D2)F_1 anti-B10.AQR) gives negligible inhibition of F*AgAb binding to B10.A(5R) FcR^+ T cells (10). As shown in Table 2 a B10.S(7R) anti-B10.HTT serum, which contains antibody against I-E subregion-controlled determinants, failed to inhibit binding of F*AgAb complexes to FcR^+ T cells from strains sharing the same I-E subregion (B10.A(5R), B10.A(3R) (Table 2). These data suggest that determinants controlled by the I-E subregion are not present on FcR^+ T cells.

TABLE 2
Inhibition of AgAb Binding to T Cells by Antisera Specific for I-region Controlled Determinants

Mouse Strain	I-Region A B J E C	% Decrease in Proportion FcR^+ T Cells after Treatment with Antisera Specific for: 2_I^k	3_I^s	$4_A^k{}_B^k{}_J^k$	5_A^b	6_J^k	$7_E^k{}_C^k{}_S^k{}_G^k$
B10.HTT	s s s k k	31	52	0	-	-	30
B10	b b b b b	-	-	7	35	3	4
B10.A(3R)b	b b k d	-	-	3	-	4	5
B10.A(4R)k	b b b b	-	-	30	0	-	-
B10.A(5R)b	b k k d	-	-	0	-	2	3

[1]See footnote 1, TABLE 1.

[2]A.TH anti-A.TL, used at 1:20 dilution.

[3]A.TL anti-A.TH, used at 1:20 dilution.

[4](B10.HTT x A.TFR5)F_1 anti-A.TL, used at 1:5 dilution.

[5]B10.A(4R) anti-B10, used at 1:5 dilution after absorption with EL-4.

[6]B10.A(3R) anti-B10.A(5R), used at 1:5 dilution.

[7]B10.S(7R) anti-B10.HTT, used at 1:5 dilution; similar results obtained at 1:20 dilution.

In contrast to its effect on FcR^+ T cells from strains 3R and 5R the B10.S(7R) anti-B10.HTT serum significantly inhibits the binding of F*AgAb complexes to B10.HTT FcR^+ T cells (Table 2). This inhibition must be due to determinants controlled by a locus mapping in the I-C, S, or G regions.

In conclusion, the data presented in this report show that determinants controlled by loci mapping in the I-A and I-C, S, or G regions are present on the population of peripheral T lymphocytes which bear an Fc receptor. Whether these determinants are controlled by the Ia-1 and Ia-3 loci, which mark the I-A and I-C subregions respectively, and whether they are shared with B lymphocytes has not been resolved. In contrast, determinants controlled by loci mapping in the I-J and I-E subregions are not detectable on the FcR^+

T cell population, which indicates either that antibodies against these determinants do not interfere with the T cell FcR or that these determinants are not expressed on the FcR^+ T cells.

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