INTERACTIONS OF Fc RECEPTORS WITH ANTIBODIES AGAINST Ia ANTIGENS AND OTHER CELL SURFACE COMPONENTS*

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Receptors for the Fc portion of the immunoglobulin molecule are expressed on a number of cells, including B lymphocytes, mast cells, polymorphonuclear cells, and macrophages (for review see 1). The biological function of these Fc receptors is in most instances only poorly understood and their chemical nature is at present unknown. Recently, Dickler and Sachs (2) reported evidence for an identity or close association of the Fc receptor of mouse B lymphocytes and alloantigens determined by the *I* region of the *H-2* complex (*I*-region-associated or Ia antigens). Binding of aggregated Ig to Fc receptors of B lymphoctyes was markedly inhibited by pretreatment of the lymphocytes with anti-Ia antibody and its Fab fragment, not however by pretreatment with antibody specific for antigens determined by the *K* or *D* regions of the *H-2* complex (anti-*H-2K* or anti-*H-2D*). Since an identity of Ia antigens and Fc receptors would have potential implications for our understanding of the mechanism of cell co-operation and of the genetic control of immune responses, we decided to further investigate this question.

Two different techniques were employed for studying Fc receceptors: (*a*) antibody-coated erythrocytes (EA)[1] rosettes (3) and (*b*) antibody-dependent cell-mediated cytotoxicity, i.e. K-cell cytotoxicity (4). In these assays, chicken erythrocytes coated with rabbit antichicken erythrocytes antibody (RACA) were used as indicator cells (for rosettes) or as target cells (for cytotoxicity).

In previous studies (5, 6) we have demonstrated that the Fc receptor of K cells is inhibited by antibodies against *H-2* antigens and against other cell surface components. This inhibition, however, proved to depend completely on an intact Fc portion of the antibody molecules and did therefore not represent a direct interaction of the Fc receptor and the antibody-combining site.

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[1] *Abbreviations used in this paper:* CIA, cytotoxicity inhibition assay; EA, erythrocytes coated with antibody; EAC, erythrocytes coated with antibody and complement; Ia antigens, I region-associated antigens; K cells, effector cells in antibody-dependent cell-mediated cytotoxicity, probably a heterogeneous population; RACA, rabbit antichicken erythrocyte antibody; R anti-BA-θ, rabbit antimouse brain-associated θ; R anti-MBLA, rabbit antimouse B lymphocyte antigen; R anti-MIg, rabbit antimouse immunoglobulin; RFC, rosette-forming cells.

In the present study we specifically tested anti-Ia antibodies for their possible interactions with Fc receptors and compared them in this respect with antibodies against other cellular antigens. We will present results which suggest that (*a*) no unique association exists between Ia antigens and the Fc receptors detected in either of the two assays, (*b*) the Fc receptor of the rosette-forming cell (RFC) and the Fc receptor of the cytotoxic K cell may not be identical.

Materials and Methods

Animals. Young adult male and female mice from inbred strains bred in this laboratory were used.

Preparation of Antisera, IgG, and F(ab')$_2$ Fragments. The various allogeneic and xenogeneic antisera were raised as described previously (5). The preparation of IgG (ammonium sulfate precipitation and DEAE-cellulose chromatography) and of F(ab')$_2$ fragments (peptic digestion of the IgG protein) has been described in detail elsewhere (6).

EA Rosette Assay. Chicken erythrocytes were sensitized with subagglutinating amounts of RACA-IgG (EA) by incubating a 2% erythrocyte suspension for 30 min at room temperature in Eagle's medium containing 1:50 diluted RACA-IgG. 50 μl of washed EA (2% suspension) was mixed with 100 μl of lymph node cells (6×10^6/ml; from popliteal, inguinal, and axillary nodes) and spun at 4°C for 3 min at 150 *g*. The cells were carefully suspended using a pasteur pipette and observed under phase contrast for the number of RFC (lymphoid cells surrounded by three or more chicken erythrocytes). For each sample about 800 lymphoid cells were screened in four separate readings and the % RFC calculated. In the inhibition assay, the lymph node cells were preincubated with the test sera at different dilutions for 30 min on ice, before the EA suspension was added.

Antibody-Dependent Cell-Mediated Cytotoxicity. The test was done as described in detail previously (5). The test conditions were adjusted to give about 50% specific ^{51}Cr release, the spontaneous ^{51}Cr release being <5%.

Results

Specific inhibition of EA-RFC and cytotoxic K cells by various antisera

EA-RFC. Fc receptor-bearing mouse lymphoid cells were detected by a rosette technique using chicken erythrocytes coated with subagglutinating amounts of rabbit IgG antibody (EA) as indicator cells. The test measured Fc receptor binding since rosette formation (*a*) was dependent on an intact Fc portion of the anti-erythrocyte antibody and (*b*) was inhibited by preincubating the lymphocytes with nonrelevant antigen-antibody complexes. The percentage of EA rosette-forming cells (EA-RFC) in different lymphoid organs (spleen, lymph node, thymus) roughly corresponded with the expected percentage of B lymphocytes.[2]

In order to test for a possible identity or association of Ia antigens and Fc receptors, rosette inhibition experiments were performed. The results, some of which are given in Table I, demonstrated specific inhibition with antisera raised between strains of completely different *H-2* haplotype (e.g. exp. 1) as well as with antisera raised in congenic strains of identical *H-2K* and *H-2D* specificities, differing only at the *I* and *S* region of the *H-2* complex (e.g. A.TH anti A.TL [7]; exp. 2). Both of these results could be due to anti-Ia antibody acitivity in the sera. However, the results of experiment 3 show that an antiserum raised in *H-2*

[2] Details about the assay and about the nature of EA-RFC will be published in: P. Halloran, V. Schirrmacher, and C. S. David. The specificity and significance of the inhibition of Fc receptor binding by anti-*H-2* sera. Manuscript submitted for publication.

TABLE I
Inhibition of EA RFC by Alloantisera

Exp.	Strain	Serum*	Principal specificity	EA-RFC‖	Inhibition
				%	%
1	BALB/c	Normal mouse serum	—	20.0 ± 2.7	
		CBA/H anti-BALB/c	Anti-*H-2* + non-*H-2*	3.3 ± 1.3	83
		BALB/c anti-CBA/H	Anti-*H-2* + non-*H-2*	18.6 ± 3.2	7
	CBA/H	Normal mouse serum	—	16.0 ± 4.0	
		CBA/H anti-BALB/c	Anti-*H-2* + non-*H-2*	14.5 ± 4.2	9
		BALB/c anti-CBA/H	Anti-*H-2* + non-*H-2*	2.0 ± 0.9	87
2	B10.A	—	—	27.5 ± 5.1	
		A.TH anti-A.TL‡	Anti-Ia	1.8 ± 1.8	94
	A.SW	—	—	19.5 ± 3.6	
		A.TH anti-A.TL‡	Anti-Ia	16.3 ± 3.1	16
3	C57BL/10	—	—	16.5 ± 3.6	
		BALB/c anti-B10.D2	Anti-Ly 4.2§	3.5 ± 0.5	79
		AKR anti-θ C3H	Anti-Thy 1.2§	18.0 ± 2.0	0
	BALB/c	—	—	15.5 ± 1.7	
		BALB/c anti-B10.D2	Anti-Ly 4.2	17.0 ± 1.3	0
		AKR anti-θ C3H	Anti-Thy 1.2	14.0 ± 1.5	10

* Serum dilution in exp. 1, 1:100; in Exp. 2 and 3, 1:20.
‡ Anti-Ia 1, 2, 3, 7 (7). B10.A carries all of these Ia specificities, A.SW none.
§ The sera were raised in *H-2* identical strains against non-*H-2* alloantigens. Ly 4.2 has been described as a B cell marker (8), Thy 1 as a T cell marker (9).
‖ Mean values ± SD from quadruplicate readings.

identical strains against a non-*H-2* alloantigen, Ly 4.2 (8), can also specifically inhibit EA-RFC. Another anti-non-*H-2* antiserum, anti-Thy 1.2, did not inhibit the rosettes. Since Ly 4.2 is primarily expressed on B cells (8) and Thy 1.2 on T cells (9), these results suggest that EA-RFC are B cells and that various sera reacting with B cells, like anti-Ia (10) and anti-Ly 4.2, lead to rosette inhibition.

Xenogeneic antisera reacting preferentially with mouse B and T cells were also tested for their ability to inhibit EA-RFC (Table II). While a rabbit antimouse brain-associated θ-antiserum (R anti-BA-θ) showed no significant inhibition (compared to normal rabbit serum), rabbit antisera against either mouse bone marrow-derived lymphocyte antigen (R anti-MBLA) or against mouse immunoglobulin (R anti-MIg) strongly inhibited EA rosette formation. The specificity of the inhibition by the polyvalent R anti-MIg was verified in several ways: (*a*) it was neutralized in presence of MIg (Table II) and of mouse myeloma proteins (V. Schirrmacher, unpublished data), (*b*) inhibition was also observed with rabbit antisera specific for distinct mouse immunoglobulin classes (V. Schirrmacher, unpublished data), and (*c*) inhibition was not observed with rabbit RFC (Table II). In summary, mouse lymphoid cells forming EA rosettes were specifically inhibited by anti-Ia, anti-Ly 4.2, R anti-MBLA, and R anti-MIg (all sera reacting with B lymphocytes), not however by anti-Thy 1 and R anti-BA θ (anti-T-cell sera).

TABLE II

Inhibition of EA RFC by Xenogeneic Antisera

Exp.	Strain	Serum‡	EA-RFC‖	Inhibition
			%	%
1	B10.A	—	15.5 ± 0.9	
		Normal rabbit serum	13.3 ± 1.8	13
		R anti-MBLA	0.5 ± 0.8	97
		R anti-BA-θ	12.3 ± 0.8	20
2	C57BL/6	—	17.1 ± 2.7	
		R anti-MIg	<1	>94
		MIg§	15.0 ± 2.8	12
		R anti-MIg + MIg§	15.5 ± 2.3	9
	Rabbit PBL*	—	11.5 ± 2.8	
		R anti-MIg	10.3 ± 2.4	10

* Rabbit lymphocytes, serving as specificity control for the rabbit antiserum, were separated from peripheral blood by the Ficoll-Isopaque technique.

‡ Serum dilution in exp. 1, 1:20; in exp. 2, 1:50.

§ 10 μg of gammaglobulin prepared from normal mouse serum by DEAE-cellulose chromatography.

‖ Mean values ± SD from quadruplicate readings.

Cytotoxic K Cells. Cytotoxic K-cell activity was tested in a system consisting of normal mouse spleen cells as a source of K cells, ^{51}Cr-labeled chicken erythrocytes as target cells and minute amounts of antitarget cell antibody (5). All the antisera which were tested in the rosette assay (Table I and II) were also tested for their ability to inhibit ^{51}Cr release in the above system. Specific inhibition of cytotoxicity was found with all the antisera which specifically inhibited EA rosettes. In addition, inhibition of cytotoxicity was observed with the antisera against mouse T cells which did not inhibit EA rosettes. These results were published recently (5). They form the basis of our cytotoxicity inhibition assay (CIA), which is a new sensitive assay for antibody against cell surface antigens.

Inhibitory potency of F(ab')$_2$ fragments of anti-Ia antibodies and R anti-MIg

Inhibition by antibody of Fc receptor binding could be due to binding of the antibody (*a*) via its binding site or (*b*) via its Fc portion (e.g. after formation of complexes with cellular antigens [6]). In order to test whether EA rosette inhibition was due to a direct interaction with the binding site of the antibody molecule (mechanism *a*), we investigated the inhibitory potency of antibodies from which the Fc portions had been removed by pepsin digestion. Fig. 1 A illustrates the results obtained with whole IgG molecules and F(ab')$_2$ fragments of anti-Ia antibodies. Both the IgG and the F(ab')$_2$ fragments showed specific inhibition of EA-RFC from strains positive for the respective Ia antigens. These results demonstrate that the inhibition of EA-RFC by anti-Ia antibody occurs via the binding site of the antibody molecule (mechanism *a*). The slight reduction in the degree of inhibition observed with F(ab')$_2$ fragments was probably not due to a loss of binding activity (see below).

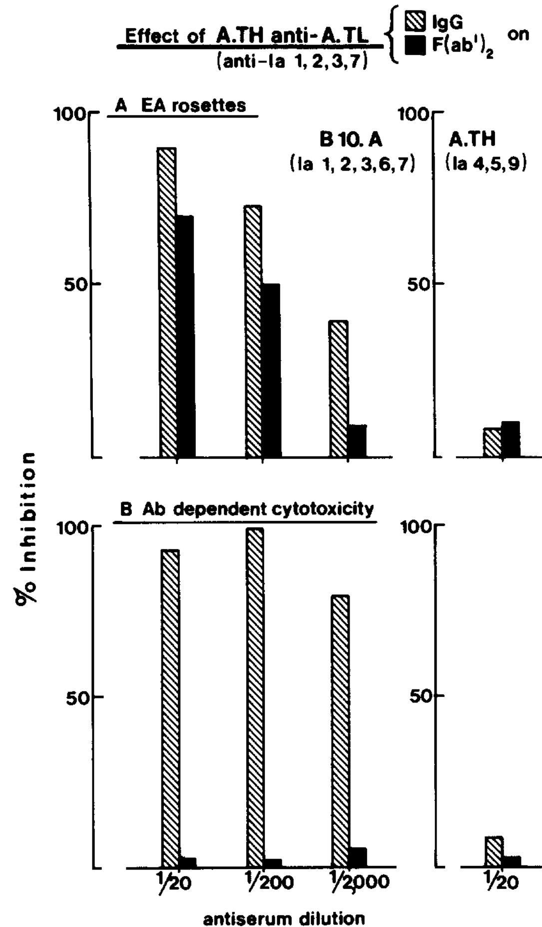

FIG. 1. Inhibition of EA-RFC (A) and of antibody-dependent cell-mediated cytotoxicity (B) by anti-Ia antibodies and their $F(ab')_2$ fragments. (A) The columns indicate the % inhibition of EA-RFC in presence of A.TH anti-A.TL (mean values from four separate experiments with lymph node cells; similar results were obtained using spleen cells). In a Student's t test, the results obtained with IgG and $F(ab')_2$ were significantly different ($P \leq 0.05$) only at the dilution of 1:2,000. (B) Spleen cells from B10.A or A.TH mice were tested for cytotoxicity against ^{51}Cr labeled antibody-coated chicken erythrocytes in absence or presence of different amounts of A.TH anti-A.TL. The % inhibition of ^{51}Cr release is indicated by the different columns.

When the same antibody preparations were tested for inhibition of K-cell activity (Fig. 1 B) strong and specific inhibition was observed when using the whole IgG antibody molecules, but no inhibition was obtained with the $F(ab')_2$ fragments. In this system anti-Ia antibodies thus inhibited only via their Fc portion (mechanism *b* above) and not via their binding sites.

We reported above that antibodies against cell surface antigen other then Ia could also inhibit EA-RFC. It was therefore of interest to study the inhibitory potency of $F(ab')_2$ fragments of such antibodies. Fig. 2 illustrates the results obtained with R anti-MIg and its $F(ab')_2$ fragment. EA-RFC were inhibited with both the intact and the digested antibody molecules (Fig. 2 A), the latter being

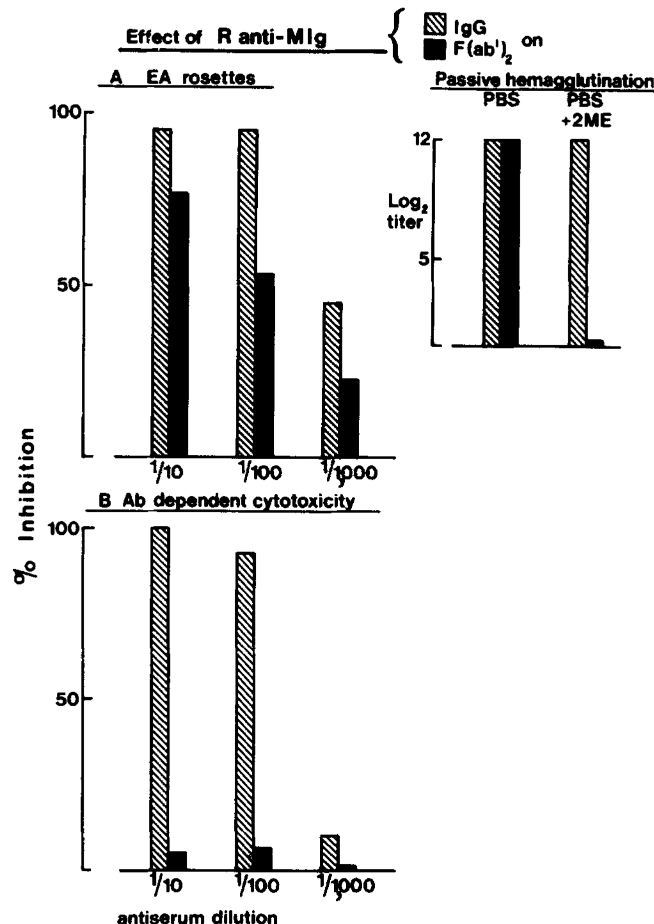

FIG. 2. Inhibition of EA-RFC (A) and of antibody dependent cell-mediated cytotoxicity (B) by rabbit antimouse Ig antibody (R anti-MIg) and its $F(ab')_2$ fragments. (A) % inhibition of EA-RFC using CBA/H lymph node cells. (Similar results were obtained using spleen cells). In a Student's t test, the differences between the results obtained with IgG and $F(ab')_2$ were significant ($0.1 \leq P \leq 0.05$). (B) % inhibition of cytotoxicity of CBA/H spleen cells against ^{51}Cr labeled antibody-coated chicken erythrocytes. The IgG and $F(ab')_2$ preparations of R anti-MIg were characterized by passive hemagglutination (upper right) using MIg-coated sheep erythrocytes (11) as indicator cells. In phosphate-buffered saline (PBS) the agglutination titers were identical, while in PBS containing 0.2 M 2-mercaptoethanol the $F(ab')_2$ fragments were reduced to two Fab fragments and did not agglutinate.

again slightly less inhibitory then the former. This was probably not due to a loss of binding activity, since in a passive hemagglutination assay (upper right) the $F(ab')_2$ fragments had the same binding activity as the whole IgG. K-cell cytotoxicity was inhibited in the presence of intact R anti-MIg antibody but not by its $F(ab')_2$ fragment (Fig. 2 B).

In summary, the effect of removing the Fc portions from inhibitory antibodies was opposite in our two assays: in the rosette assay the $F(ab')_2$ fragments could still inhibit while in the cytotoxicity test they could not. The inhibition observed in the two systems must therefore be mediated by different mechanisms. In

neither of the systems could we find any evidence for a unique association of Ia antigens and Fc receptors.

Discussion

We have shown that antibodies with a wide variety of specificities inhibit two different types of Fc receptor-dependent assays. In order to be able to draw conclusions from these data with respect to possible associations between the Fc receptor and Ia or other antigens, we must consider the mechanism of inhibition observed in each of the assays.

EA-RFC were specifically inhibited by various antisera reacting with B cells (i.e. anti-Ia, anti-Ly 4.2, anti-MBLA, antimouse Ig). This inhibition seemed to be due to direct binding of the antibody molecules via their binding sites to the RFC, since the $F(ab')_2$ fragments were specifically inhibitory. Recently we have demonstrated that both the IgG and the $F(ab')_2$ preparations of anti-*H-2K* and anti-*H-2D* antibodies also produce strong specific inhibition of EA rosette formation.[2] Since antibodies against apparently unrelated antigens (e.g. Ia, mouse Ig, Ly 4.2, *H-2K* etc.) inhibited EA rosettes, they probably did not all bind to the Fc receptor itself but bound rather to different sites on the surface of the RFC. Nevertheless, these binding reactions seemed to have a specific effect on the Fc receptor. With rosettes formed via the complement receptor (EAC) no inhibition occurred with either alloantisera (2,12) or specific anti-Ia antisera (A. Arnaiz-Villena, unpublished results). Thus, in the EA rosette assay no evidence was found which would support the assumption of a unique association of the Fc receptor with Ia antigens. We recently obtained some additional evidence which would rather be against such an assumption: in certain tissues EA-RFC were found which were not inhibitable by anti-Ia antibodies.[2]

The mechanism of the inhibition of K-cell cytotoxicity by antiserum against antigens in the cell population containing the K cells has been analyzed in detail previously (6). In brief, it involves binding of the antibody to cell membrane antigens and the interaction of its Fc portion with the Fc receptor of the K cell. $F(ab')_2$ fragments therefore had no inhibitory effect in this system. In the present report we have shown that anti-Ia antibodies do not behave differently from other antibodies in the cytotoxicity inhibition assay: while the intact antibody molecules strongly and specifically inhibited K-cell activity from strains positive for the respective Ia antigens, the $F(ab')_2$ fragments were completely devoid of inhibitory activity. Similar results have been obtained with several different anti-Ia antisera. In further experiments we could show that pretreatment of the cytotoxic cells with excess $F(ab')_2$ anti-Ia antibody had no effect on their cytotoxicity and also no effect on the inhibition of their cytotoxicity by unrelated complexes. We conclude from these experiments that Fc receptors of K cells are not associated with Ia antigens.

The results obtained in both our assays differ from those obtained in an aggregated Ig-binding assay, where anti-Ia antibodies were reported to be the only antibodies inhibiting the reaction (2). Reasons for these discrepancies are at present not known. Both the aggregated Ig-binding assay and the EA rosette assay seem to measure Fc receptors on B lymphocytes.

With regard to EA rosettes and cytotoxic K cells we have preliminary data

indicating different characterisitcs of the Fc receptors involved. This seems already obvious from the different effect of the removal of the Fc portion from the inhibiting antibodies in the two assays (see Figs. 1 and 2). These qualitative differences in the functional behavior of Fc receptors may imply structural and/or functional heterogeneity of Fc receptors.

Summary

Two Fc receptor-dependent tests were investigated to study the question of a relationship between Fc receptors and known cell surface antigens, in particular *I* region-associated (Ia) antigens: (*a*) a rosette assay with antibody-coated erythrocytes (EA) as indicator cells and normal mouse lymphoid cells as source of rosette-forming cells, and (*b*) a cytotoxicity test with antibody-coated erythrocytes as target cells and normal mouse spleen cells as a source of cytotoxic cells (K cells).

EA rosettes were specifically inhibited by antibodies reacting with Ia antigens. Various other antisera reacting with antigens on B lymphocytes, like anti-Ly 4.2 (raised in *H-2* identical mice), rabbit antimouse B-cell serum, or rabbit antimouse immunoglobulin, also specifically inhibited the rosettes. No inhibition occurred in the presence of allogeneic or xenogeneic antisera reacting with T lymphocytes. K-cell cytotoxicity was specifically inhibited by each of the antisera (reacting with either B cells or T cells).

$F(ab')_2$ fragments of anti-Ia antibodies could still specifically inhibit EA rosettes but they could not inhibit K-cell cytotoxicity. Similar results were obtained with $F(ab')_2$ fragments of anti-immunoglobulin antibodies. These results indicate that the mechanism of inhibition of Fc receptors in the two tests was different. In neither of the tests could we find any evidence for a unique association between the Fc receptors and Ia antigens. The Fc receptors on K cells did not seem to be associated at all with Ia antigens.

This publication is dedicated to Professor Dr. Günther Weitzel on the occasion of his 60th birthday. We would like to thank Dr. Hilliard Festenstein for his help and encouragement and Mrs. Barbara Reynolds for her expert technical assistance.

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