RELATIONSHIP BETWEEN Fc RECEPTORS, ANTIGEN-BINDING SITES ON T AND B CELLS, AND H-2 COMPLEX-ASSOCIATED DETERMINANTS*

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B lymphocytes bear on their membranes a receptor for the Fc piece of certain classes of immunoglobulin (Ig) molecules (1-5). The bond between Ig and receptor is stable only when Ig is complexed to antigen (3) or aggregated (1) and, in man, both immune complex and aggregated Ig bind to the same receptor (5). The Fc receptor is distinct from the specific antigen-binding site (Ig determinant) on B cells (3, 5) and from the C3 receptor (6). Binding of Ig is not dependent on complement (C) (1-3), pH, temperature, or divalent cations (1); it is inhibited by antiserum specific for Ia antigens associated with the Ir region of the H-2 complex (7), but not by polyvalent anti-Ig sera (4, 7). The receptor appears to be a trypsin-resistant protein or glycoprotein (5) and to be involved in antibody-dependent lymphocyte cytotoxicity (5).

The present studies were carried out to investigate the relationship between the Fc receptor, the alloantigens determined by the H-2 complex, and the specific antigen-binding sites on lymphocytes. It appears that, on B lymphocytes, some alloantigens coded by a region of the H-2 complex lie in close proximity to the Fc receptor but not to the specific antigen-binding (Ig) site. By contrast, these H-2-determined alloantigens seem to be closely associated with the antigen-specific binding receptor on T lymphocytes.

Materials and Methods

Animals. Male and female mice of the highly inbred CBA/H/WEHI, AKR/J, and C57BL/6J strains were used. The latter two strains were obtained from Jackson Laboratories, Bar Harbor, Maine, and the former bred from pedigree stock at the Walter and Eliza Hall Institute, Melbourne, Australia. Their origin and maintenance have been previously described (8). Athymic nude mice (nu/nu) were kindly provided by Dr. Margaret Holmes of the Walter and Eliza Hall Institute.

Operative Procedures. Thymectomy was performed in 4- to 7-wk old CBA mice as described by Miller (9). The mediastinum was examined at autopsy and any mice with thymus remnants discarded.

Cell Suspensions. Single cell suspensions of thymus, spleen, and lymph nodes were obtained by teasing tissues through an 80 mesh stainless steel sieve into cold Eisens’s solution as previously described (8). Cells were washed three times before use.

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Removal of Dead Cells. Dead cells were removed from suspensions by the method of von Boehmer and Shortman (10).

Viability Counts. Cell viability was estimated by the dye exclusion technique, as routinely done in this laboratory (8).

Antigens. Fowl immunoglobulin G (FyG) was obtained from chicken serum by the method of Miller and Warner (11). Before iodination it was absorbed extensively against mouse lymphocytes to remove nonspecific anti-mouse leukoagglutinating activity. Sheep erythrocytes (SRBC) and horse erythrocytes (HRBC) were collected and stored in Alsever’s solution as previously described (8).

Injections. Cell suspensions and antigens were injected intravenously (i.v.) or intraperitoneally (i.p.) as indicated in the text.

Irradiation. Intact or thymectomized mice were exposed to total body irradiation in a clinical teletherapy machine (Theratron 75, Atomic Energy Commission of Canada Ltd., Canada) between opposed vertical fields under conditions of minimum back scatter. The dose used was 750-800 R at a rate of 80 R/min. In the case of thymectomized mice, irradiation was performed 2-4 wk post-operatively and reconstitution achieved by injecting 3-5 x 10^4 syngeneic bone marrow cells i.v. within 3 h. These thymectomized, irradiated marrow protected (TxBM) mice behave essentially as do T-cell-deprived mice (12). They were given polymyxin B (10^4 IU/liter) and neomycin (10 mg/liter) in the drinking water.

Immunization. Mice were primed to HRBC by injection of 5 x 10^6 cells i.v. For immunization with FyG, the antigen was alum precipitated as previously described (8) and 500 μg given i.p. together with 2 x 10^6 killed pertussis organisms (Commonwealth Serum Laboratories, Melbourne, Australia). Cells from such donors were used 4-8 wk postpriming.

Preparation of Anti-θ Serum. Anti-θ C3H serum was raised in AKR/J mice according to the method of Reif and Allen (13). After incubation at 56°C for 30 min, the potency of each batch was tested by cytotoxicity against CBA thymus and lymph node cells. Agarose-absorbed guinea pig serum provided the source of C. Anti-θ AKR serum was prepared in a similar manner except that AKR thymus cells were injected into CBA recipients. For preparation of T-cell-depleted populations, 1 ml of anti-θ C3H serum was added to 10^9 spleen cells at a concentration of 25 x 10^6 cells/ml. After incubation for 30 min at 37°C, the cells were washed twice and exposed (5 x 10^6 cells/ml) to C diluted 1 in 6 for a further period of 30 min at 37°C.

Preparation of Antiser. Anti-H-2 sera were prepared by repeated injections of CBA and C57BL/6J mice with lymphocytes from C57BL/6J and CBA donors, respectively, according to the schedule previously reported (8). Each batch was heat inactivated for 30 min at 56°C and assessed for cytotoxic activity by dye exclusion. Samples from a single batch of C57BL/6J anti-CBA serum were extensively absorbed (four to five times) either with spleen cells from TxBM CBA mice or with CBA thymus cells until no residual anti-H-2k activity was demonstrable.

Preparation of Aggregated Human Gamma Globulin (Agg). Human gamma globulin was obtained from the Commonwealth Serum Laboratories as a solution containing 160 mg/ml. The IgG fraction was further purified by gel filtration through Sephadex QAE 50 (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) and absorbed extensively against mouse thymus cells. Aggregation was performed as described by Dickler and Kunkel (1).

Fluorescein Isothiocyanate (Fl)-Labeled Reagents. A polyvalent rabbit antimouse IgG was prepared as previously described (14). The IgG fraction was separated by gel filtration through QAE Sephadex 50. Polyvalent Fl-rabbit antihuman Ig and Fl-sheep antirabbit Ig reagents were obtained from the Wellcome Research Laboratories, Beckenham, Kent, England.

Preparation of Iodinated Reagents. FyG and Agg were iodinated with 125I (The Radiochemical Centre, Amersham, England, cat. no. IMS3) according to the method of Byrt and Ada (15). The sp act of 125I-labeled FyG for antigen suicide was 150 μCi/μg and for autoradiographic studies 40-50 μCi/μg. The sp act of 125I-labeled Agg was 150-200 μCi/μg.

Preparation of Anti-H-2k Fab Fragment. The IgG fraction of anti-CBA (anti-H-2k) antisera was obtained as previously described (4). The Fab fragments were prepared from them according to the

1 Abbreviations used in this paper: Agg, heat-aggregated human Ig; BSA, bovine serum albumin; FCS, fetal calf serum; FyG, fowl immunoglobulin G; Fl, fluorescein isothiocyanate; HRBC, horse erythrocytes; PBS, phosphate-buffered saline; PFC, plaque-forming cells; TxBM, thymectomized, irradiated, marrow protected.
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Method of Porter (16). Samples of these were extensively absorbed (four to five times) with either CBA thymus cells or spleen cells from TxBM CBA mice until no residual cytotoxic anti-H-2k activity was demonstrable.

Detection of Fc Receptors on B Cells by 125I-Labeled Immune Complexes. Fc receptors on B cells were detected with anti-FyG-125I-labeled FyG as previously described (3). For inhibition studies 2 × 10⁹ lymphoid cells were incubated with 50 μl antisera or anti-H-2k Fab (1 mg/ml) in 0.2 ml Eisen’s solution for 60 min at 4°C. After washing twice, the cells were exposed to labeled immune complexes under standard conditions and smears prepared for autoradiography.

Separation of T cells. Lymphoid cell suspensions enriched for T cells were obtained by passing normal CBA spleen cells through anti-Ig-coated columns according to the method of Campbell and Grey (17). The efficiency of separation was tested by treating the final cell suspensions with Fl-rabbit IgG antimouse Ig to estimate the number of residual B cells. Usually less than 3% of cells in the column effluent stained with the fluorescent reagent.

Incubation of Cells with Fl-Labeled Reagents. Ig-bearing lymphocytes were detected by a sandwich technique using rabbit IgG antimouse Ig followed by Fl-sheep antirabbit Ig essentially as previously described (14). Labeled cells were counted using a Leitz orthoplan microscope (E. Leitz, Inc., Rockleigh, N. J.). For the detection of Agg-binding lymphocytes, 2 × 10⁹ lymphoid cells were incubated with 40 μl Agg (5 mg/ml) in 0.2 ml phosphate-buffered saline (PBS) containing 2% bovine serum albumin (BSA) and 1.5 mM sodium azide for 30 min at 4°C. The cells were washed twice and then exposed to 25 μl Fl-rabbit IgG antihuman Ig in 0.2 ml PBS-BSA for a further period of 30 min at 4°C. Labeled cells were counted as described above.

Suicide Techniques. Antigen-induced suicide of T and B cells was carried out by a modification of the method of Basten et al. (18). For T cells, 10 μg 125I-labeled FyG (sp act 150 μCi/μg) was added to 5 × 10⁹ anti-δ serum-treated spleen cells from mice primed to FyG and HRBC for 30 min at 4°C. The supernate was removed and 10⁸ purified T cells from the same source added. The resultant mixture was incubated for 1 h at 37°C in Hapes-buffered RPMI 1640 and then overnight in the cold in the same medium containing 10% fetal calf serum (FCS, Commonwealth Serum Laboratories). Excess radioactivity was removed by centrifugation through two discontinuous FCS gradients. B-cell suicide was carried out by incubating anti-δ serum-treated spleen cells primed to FyG and HRBC with 125I-labeled FyG entirely in the cold as previously described (18). For inhibition studies, purified T cells or anti-δ serum-treated spleen (B) cells were incubated with anti-H-2k Fab (1 mg/10⁸ cells) for 3 h at 4°C. After washing they were exposed to 125I-labeled FyG as described above. Control cells were treated in the same way except that 125I-labeled FyG was used instead. The suicide effect was assayed by injecting combinations of T cells and B cells i.v. into heavily irradiated mice together with 5 × 10⁸ HRBC and 100 μg FyG per mouse.

Agg-induced suicide was carried out as follows: spleen cells from mice primed to FyG and HRBC were exposed to 125I-labeled Agg at a concentration of 10 μg/10⁸ cells/ml for 1 h at 4°C. Excess aggregates were removed by centrifugation through discontinuous FCS gradients and the cell pellet resuspended in rabbit antihuman Ig serum at a concentration of 0.2 ml/10⁸ cells in 2 ml of Hapes-buffered RPMI 1640. The mixture was then incubated for a further 30 min in the cold, washed, and placed at 4°C for 18 h in the same medium containing 10% FCS. Control cells were treated with 125I-labeled Agg under identical conditions.

For inhibition studies spleen cells were preincubated with anti-H-2k Fab (3 mg/10⁸ cells) for 3 h at 4°C. After washing they were then incubated with 125I-labeled Agg as described above. The suicide effect was assayed in the same way as for antigen-induced suicide except that 5 × 10⁸ SRBC were injected as well as HRBC and FyG.

Detection of Antibody-Forming Cells. Antibody-forming cells were detected as described before (8, 11).

Statistical Analyses. These were performed as described in a previous paper (9).

Results

Effect of Pretreatment with Anti-H-2k Fab on 125I-Labeled FyG-Induced Suicide of T and B cells. The relationship between H-2-associated antigens and antigen-binding sites on T and B cells was examined in a functional assay by the suicide technique. For this purpose, purified populations of lymphoid cells were
incubated with highly substituted $^{125}$I-labeled FyG and transferred into heavily irradiated mice together with FyG and a control antigen, HRBC (Table I). T cells were obtained by passage of normal spleen cells through anti-Ig-coated columns and B cells by treatment of spleen cells from mice primed to FyG and HRBC with anti-θ serum and C. In preliminary experiments incubation of $^{125}$I-labeled FyG with T cells failed to result in abrogation of the adoptive FyG response even when the procedure was carried out at 37°C. Suicide was, however, achieved if the radioactive antigen was presented to the T cells on anti-θ serum-treated spleen cells at a ratio of 1:20. They were then injected together with $5 \times 10^8$ primed B cells (see text).

Table I

| Group | Cells given* | No. of irradiated recipients‡ | Indirect PFC per spleen at 7 days |
|-------|--------------|-------------------------------|----------------------------------|
|       |              |                               | Anti-FyG | Anti-HRBC |
| 1     | $5 \times 10^8$ B cells | 7 | 760 (1.17)§ | 1030 (1.12) |
| 2     | $10^7$ T cells | 8 | 35 (2.24) | 40 (2.35) |
| 3     | $10^7$ T cells + $5 \times 10^8$ B cells | 8 | 13,280 (1.09) | 16,240 (1.12) |
| 4     | $10^7$ T cells treated with $^{125}$I-labeled FyG + $5 \times 10^8$ B cells || 8 | 1,570 (1.12) | 19,380 (1.10) |
| 5     | $10^7$ T cells + $5 \times 10^8$ B cells treated with $^{125}$I-labeled FyG | 8 | 1,410 (1.19) | 22,740 (1.10) |
| 6     | $10^7$ T cells treated with anti-H-2k Fab followed by $^{125}$I-labeled FyG + $5 \times 10^8$ B cells | 8 | 15,020 (1.13) | 18,270 (1.09) |
| 7     | $10^7$ T cells + $5 \times 10^8$ B cells treated with anti-H-2k Fab followed by $^{125}$I-labeled FyG | 7 | 440 (1.20) | 16,470 (1.07) |
| 8     | $10^7$ spleen cells primed to FyG and HRBC | 6 | 262,460 (1.08) | 263,640 (1.09) |

* T cells obtained by passage of normal spleen cells through anti-Ig-coated columns. B cells obtained by treatment of FyG- and HRBC-primed spleen cells with anti-θ serum and C.

† Each recipient given 100 μg fluid FyG i.p. and $5 \times 10^8$ HRBC i.v.

§ Geometric means and standard errors.

The $125$I-labeled FyG in the presence of anti-θ-treated FyG-primed spleen cells at a ratio of 1:20. They were then injected together with $5 \times 10^8$ primed B cells (see text).
contrast, no such effect was observed when B cells were pretreated with anti-H-2k Fab (group 7). Taken together these findings indicate that H-2 (?Ir)-associated determinants are in close proximity to the specific antigen-binding sites on T cells but not on B cells.

**Effect of Pretreatment with anti-H-2 Serum on the Binding of Immune Complexes to the Fc Receptor on B cells.** The previous experiment indicated that H-2-associated antigens were not in close proximity to the region of the antigen-binding (Ig) determinants on B cells. The question therefore arose whether other sites, such as the Fc receptors, were more closely linked to H-2 determinants since a role for the Fc receptor had been postulated in the regulation of B-cell activity (3, 7). Spleen cells from CBA/H/WEHI (H-2k) mice were incubated with anti-H-2 serum directed against H-2k or H-2b determinants, washed, and then labeled with anti-FyG 

\[ ^{125}I \text{-labeled FyG} \] in the standard manner. As shown in Table II, pretreatment with anti-H-2k serum reduced the binding of immune complexes from 37% to less than 1%. The specificity of inhibition was examined in two ways. First, H-2k spleen cells were incubated with anti-H-2b serum; no reduction in labeled cells was observed. Second, a mixture of equal numbers of H-2k and H-2b spleen cells were exposed to anti-H-2k or anti-H-2b serum; under these conditions the great majority of available Fc receptors were blocked by either antiserum. In other words inhibition of binding was nonspecific.

The blocking capacity of the anti-H-2 sera was completely abrogated by absorption with spleen cells from TxBM mice of appropriate H-2 specificities. In

| Source of spleen cells | Pretreatment | Splenic lymphocytes displaying Fc receptors* |
|-----------------------|--------------|--------------------------------------------|
| **CBA/H/WEHI**        | Normal       | 37 (29-42)†                               |
| **C57BL/6J**          | Normal       | 38 (31-44)                                |
| **CBA/H/WEHI**        | Anti-H-2k    | <1                                         |
| **C57BL/6J**          | Anti-H-2k    | 36 (31-39)                                |
| **CBA/H/WEHI + C57BL/6J** | Anti-H-2k    | 5 (2-7)                                   |
| **CBA/H/WEHI**        | Anti-H-2k (thymus absorbed)§ | 4 (3-6)                                   |
| **CBA/H/WEHI**        | Anti-H-2k (TxBM spleen absorbed)‖ | 33 (29-37)                               |
| **C57BL/6J**          | Anti-H-2b    | 2 (0.5-4)                                 |
| **CBA/H/WEHI**        | Anti-H-2b    | 39 (33-44)                                |
| **CBA/H/WEHI + C57BL/6J** | Anti-H-2b    | 7 (5-8)                                   |
| **C57BL/6J**          | Anti-H-2b (thymus absorbed)§ | 9 (6-11)                                  |
| **C57BL/6J**          | Anti-H-2b (TxBM spleen absorbed)‖ | 34 (30-37)                                |

* Figures obtained from one experiment. Another gave similar results.
† Range of values is given in parentheses.
§ Antisera absorbed with thymus cells until no cytotoxic activity was demonstrable against spleen cells bearing appropriate H-2 antigens.
‖ Antisera absorbed with TxBM spleen cells until no cytotoxic activity was demonstrable against spleen cells bearing appropriate H-2 antigens.
contrast, repeated absorptions with thymus cells had much less effect. For example, thymus-absorbed anti-H-2k serum retained the ability to inhibit the binding of immune complexes to H-2k B cells suggesting that it contained antibody activity against some determinants on the B cell other than those coded by the K or D end of the H-2 complex (Table II).

**Effect of Pretreatment with Anti-H-2 Fab Fragments on the Binding of Immune Complexes to the Fc Receptor on B Cells.** The nonspecificity of blockade by anti-H-2 serum did not exclude the possibility of specific binding to some determinants in the region of the Fc receptor sites. To examine this more critically the Fab fragment of anti-H-2k serum was used. It can be seen from Table III that access of immune complexes to Fc receptors was as effectively blocked by anti-H-2k Fab as by the intact antiserum. Inhibitory activity was again removed by absorption with H-2k spleen cells from TxBM mice but not with thymus cells. Furthermore, when equal numbers of H-2k and H-2b spleen cells were exposed to anti-H-2k Fab no more than 50% inhibition was observed which was consistent with specific blockade.

**Effect of Pretreatment with Anti-θ Serum on the Binding of Immune Complexes to the Fc Receptor of B cells.** Inhibition of immune complex binding to Fc receptors by treatment with reagents known to react with other determinants (i.e., H-2) on the B cell raised the question whether an antiserum directed against different cells (e.g., T cells) could have the same effect. Spleen and thymus cells from CBA/H/WEHI mice were incubated separately or in combination with anti-θ C3H serum or anti-θ AKR serum under varying conditions. As shown in Table IV, prior exposure of a mixture of spleen and thymus cells to anti-θ C3H serum for 30 min at 37°C resulted in a marked reduction in the number of Fc-positive cells compared with the control population treated with anti-θ AKR serum (21-1%). If the procedure was carried out at 4°C in the

| Source of spleen cells | Pretreatment | Splenic lymphocytes displaying Fc receptors* % |
|-----------------------|--------------|-----------------------------------------------|
| CBA/H/WEHI            | Normal mouse serum          | 40 (38-43)†                               |
| CBA/H/WEHI            | Anti-H-2k Fab               | 5 (3-6)                                       |
| CBA/H/WEHI            | Anti-H-2k Fab (thymus absorbed) | 10 (8-13)                                     |
| CBA/H/WEHI            | Anti-H-2k Fab (TxBM spleen absorbed) | 32 (30-35)                                    |
| CBA/H/WEHI + C57BL/6J§ | Anti-H-2k Fab               | 19 (17-23)                                     |
| CBA/H/WEHI            | Anti-H-2k serum             | 1 (0.5-2)                                     |
| CBA/H/WEHI + C57BL/6J§ | Anti-H-2k serum             | 6 (4-8)                                       |

* Figures obtained from one experiment. Another gave similar results.
† Range of values is given in parentheses.
§ Cells mixed at a ratio of 1:1.
presence of 1.5 mM sodium azide, a comparable reduction in cells labeled with immune complexes occurred. A similar but less marked trend was observed with spleen cells alone. Thus 8% of cells (compared with 39% in the control) bound complexes when incubation with anti-\( \theta \) C3H serum was carried out at 37°C. Furthermore this only rose to 25% if the temperature was reduced to 4°C and 1.5 mM sodium azide added. In other words, approximately \( \frac{1}{3} \) (36%) of B cells or 19% of the total lymphoid cell population could theoretically be "\( \theta \)-positive".

**Binding of Agg to Mouse Lymphoid Cells.** Agg was prepared by the method reported for the detection of Fc receptors on human B cells by Dickler and Kunkel (1). Incubation of mouse spleen cells with this reagent followed by F1-sheep antihuman Ig resulted in labeling of 45% of cells (Table V). Binding was inhibited by pretreatment with excess mouse IgG or immune complexes suggesting that Agg was indeed detecting an Fc receptor. To establish whether the Fc receptor was present on B cells or T cells, lymphoid cell populations enriched or depleted of B cells were exposed to Agg F1-sheep antihuman Ig. The results shown in Table V strongly suggest that B cells were selectively labeled by this technique.

### Table IV

**Inhibition of Binding of Immune Complexes to B cells by Anti-\( \theta \) Serum**

| Source of cells | Pretreatment with mouse serum | Conditions of pretreatment | Lymphocytes displaying Fc receptors* |
|-----------------|-------------------------------|-----------------------------|-------------------------------------|
| Spleen          | Anti-\( \theta \) AKR         | 37°C                        | 39 (34-43)‡                        |
| Thymus          | Anti-\( \theta \) AKR         | 37°C                        | 1 (0-2)                            |
| Spleen + thymus | Anti-\( \theta \) AKR         | 37°C                        | 21 (18-26)                         |
| Spleen + thymus | Anti-\( \theta \) C3H          | 37°C                        | 1 (0-2)                            |
| Spleen + thymus | Anti-\( \theta \) C3H          | 4°C + sodium azide          | 3 (1-4)                            |
| Spleen          | Anti-\( \theta \) C3H          | 37°C                        | 8 (5-12)                           |
| Spleen          | Anti-\( \theta \) C3H          | 4°C + sodium azide          | 25 (22-27)                         |

* Figures from one experiment. Another gave similar results.
‡ Range of values is given in parentheses.

### Table V

**Selective Binding of Agg to B Cells**

| Cell source | Lymphoid cells binding Agg* |
|-------------|-----------------------------|
| Normal spleen cells | 45 (30-57)‡ |
| Spleen cells pretreated with excess mouse IgG (15 mg/ml) | 11 (9-14) |
| Spleen cells pretreated with anti-FyG-FyG | 14 (11-16) |
| TxBM spleen cells | 69 (62-76) |
| Nude spleen cells | 8 (60-88) |
| Spleen cells after anti-\( \theta \) serum and C treatment | 74 (68-78) |
| Spleen cells after passage through anti-Ig-coated column | <1 |
| Thymus cells | 3 (1-7) |

* Figures obtained from one experiment. Another gave similar results.
‡ Range of values is given in parentheses.
Selective Inactivation of B Cells by Treatment with $^{125}$I-labeled Agg. The capacity of Agg to bind to B cells provided a potential method for the selective elimination of B cells from a mixed lymphoid cell population via their Fc receptors. CBA/H/WEHI spleen cells from mice primed to FyG and HRBC were therefore incubated with highly substituted $^{125}$I-labeled Agg followed by rabbit antihuman Ig serum and transferred into heavily irradiated mice together with FyG, HRBC, and SRBC. Control cells were treated with $^{125}$I-labeled Agg under identical conditions. As shown in Table VI, the PFC response to all three antigens was markedly reduced when compared to that of control cells (group 2 vs. group 1). Abrogation of direct as well as indirect PFC to SRBC implied that the Fc receptor is expressed on Bμ and Bγ cells. Addition of a small number (2.5 x 10⁸) of B cells, but not of T cells, from the original spleen cell population

**Table VI**

Selective Suicide of B Cells by $^{125}$I-Labeled Agg: Inhibition by Pretreatment with anti-H-2k Fab

| Group | Cells given* | No. of irradiated recipients† | PFC per spleen at 7 days | Anti-SRBC | Indirect anti-HRBC | Indirect anti-FyG |
|-------|--------------|-------------------------------|--------------------------|-----------|-------------------|-------------------|
|       |              |                               | Direct                  | Indirect  |                   |                   |
| 1     | $10^7$ spleen cells + $^{125}$I-labeled Agg | 8                             | 5,200(1.06) 27,200(1.03) 200,160(1.05) 156,550(1.05) |
| 2     | $10^7$ spleen cells + $^{125}$I-labeled Agg | 7                             | 20(2.23) 610(1.29) 2,450(1.18) 3,690(1.11) |
| 3     | $10^7$ spleen cells + $^{125}$I-labeled Agg + 2.5 x 10⁸ B cells | 7                             | 1,120(1.11) 9,080(1.07) 57,990(1.04) 47,400(1.03) |
| 4     | $10^7$ spleen cells + $^{125}$I-labeled Agg + 2.5 x 10⁸ T cells | 7                             | 50(1.95) 880(1.24) 2,830(1.13) 4,270(1.08) |
| 5     | $10^7$ spleen cells treated with anti-H-2k Fab + $^{125}$I-labeled Agg | 7                             | 3,770(1.05) 22,680(1.05) 169,930(1.04) 125,190(1.06) |
| 6     | $5 \times 10^8$ B cells alone | 8                             | 30(2.48) 190(2.41) 990(1.22) 1,710(1.16) |
| 7     | $5 \times 10^8$ T cells alone | 8                             | 10(2.30) 30(2.06) 1,530(1.10) 1,620(1.14) |

P values between groups. Direct PFC to SRBC: 1 cf. 2, <0.001; and 1 cf. 5, NS. Indirect PFC to SRBC: 1 cf. 2, <0.001; and 1 cf. 5, NS. PFC to HRBC: 1 cf. 2, <0.001; and 1 cf. 5, NS. PFC to FyG: 1 cf. 2, <0.001; and 1 cf. 5, NS.

* Spleen cells primed to FyG and HRBC. B cells obtained by treatment of FyG- and HRBC-primed spleen cells with anti-θ serum and C. T cells obtained by passage of FyG- and HRBC-primed spleen cells through anti-Ig-coated columns.

† Each recipient given 100 μg fluid FyG i.p. and $5 \times 10^8$ HRBC and SRBC i.v.

§ Geometric means and standard errors.
resulted in partial restoration of antibody production (groups 3 and 4). This technique therefore appeared to provide a functional assay for examining the relationship of H-2-linked antigens and Fc receptors on B cells. For this purpose the same CBA spleen cell suspension was preincubated with anti-H-2k Fab before exposure to 125I-labeled Agg. It is apparent from Table VI (group 5) that almost complete abrogation of the suicide effect was achieved thus confirming the notion of a close association between the Fc receptor and some antigens, on the B-cell membrane, coded by the H-2 complex.

Discussion

In the present study an investigation was made of the relationship between H-2-determined alloantigens, specific antigen-binding receptors on B and T lymphocytes, and Fc receptors on B lymphocytes. Ig complex binding to Fc receptors was inhibited by various antisera, including anti-H-2 sera. When a mixed population of H-2k and H-2b cells were exposed to either anti-H-2k or anti-H-2b sera, most of the available Fc receptors were blocked by either antiserum (Table II). This lack of specificity of inhibition implied that H-2 complexes formed at the cell surface during the incubation procedure and attached to the Fc receptor. The question therefore arose whether B cells might bind immune complexes formed by interaction of antibodies directed against other surface components or against components present on other cell types in the same population, e.g., T cells. This indeed proved to be the case since anti-θ C3H serum produced similar inhibitory effects (Table IV). These findings have important practical implications. First, abrogation of a response by pretreatment of cells with anti-θ serum does not necessarily imply that the response is T-cell dependent, unless restoration is achieved by addition of purified T cells. Thus, antibody-dependent cytotoxicity, which is known to be inhabitable by immune complexes (19), could under certain circumstances be anti-θ serum sensitive. Second, the use of IgG rather than Fab fragments in studies of cell membranes may lead to artifactual labeling of B cells via the Fc receptors. Thus, the assumption that a θ-positive cell is a T cell may not be correct unless the same cell is also shown to be Ig negative and C3 negative. Furthermore, other cells with Fc receptors such as macrophages might be labeled with anti-θ antibody due to shedding of θ-antigen containing complexes from T lymphocytes in the same cell suspension. Indeed we have found that, in preparations of activated thymus cells from spleens of irradiated mice, some small mononuclear cells which had ingested latex particles were θ-positive (J. F. A. P. Miller and A. Basten, unpublished data).

The nonspecificity of blockade of Fc receptor by anti-H-2 antibody does not necessarily exclude the possibility that specific binding to some component of the Fc receptor or adjacent membrane might have occurred. To determine this, the Fab fragment of an anti-H-2k antibody was used. It inhibited Ig complex binding to B cells from CBA (H-2k) mice (Table III). Blocking of the Fc receptor was demonstrated not only in binding assays (with radiiodinated immune complexes) but also in functional assays using an adoptive transfer system. Selective suicide of B cells was evident after pretreatment with 125I-labeled Agg and protection from this effect was readily achieved by exposure to an anti-H-2 Fab
reagent of appropriate specificity (Table VI). Interestingly, the blocking activity of the anti-\(H-2k\) Fab reagent could be removed by absorption with \(H-2k\) spleen cells from TxBM mice but not with \(H-2k\) thymus cells (Table II). The implications are that antibodies responsible for blocking the Fc receptor had specificities for determinants on the B-cell membrane distinct from those coded by the \(K\) or \(D\) ends of the \(H-2\) complex, and either absent from, or poorly represented on, thymus cells. These results are in general agreement with those of Dickler and Sachs (7) who showed that antisera specific for Ia antigens inhibited the binding of Ig complexes to mouse B lymphocytes. It is not clear whether the Ia antigens and Fc receptors are identical structures. It appears, however, that some Ia antigens must be distinct from Fc receptors since capping the Fc receptor directly with immune complexes did not result in capping of the Ia antigens as detected by a radio-iodinated anti-Ia reagent and autoradiography (J. F. A. P. Miller and A. Basten, unpublished data).

Exposure of lymphoid cells to \(^{125}\)I-labeled Agg was successful in achieving B-cell but not T-cell suicide (Table VI). It appears that the type of Agg which were used in these experiments bind to B cells, not to T cells. This supports our previous demonstration that B cells, not T cells, could bind immune complexes (3) and be selectively removed, after incubation with antibody, by a column of beads coated with the appropriate antigen (20). Although in accordance with the results obtained by Dickler and Sachs (7) who used a similar method of Agg preparation, it is not in agreement with the findings of other investigators (e.g., 21) demonstrating Fc receptors on T cells. These discrepancies may be accounted for by variations in the sensitivities of the assays, different types of Ig reagents used, or differential expression of Fc receptors during certain stages of lymphocyte differentiation. Alternatively there may exist more than one type of Fc receptor on one class of lymphocytes (22).

Pretreatment of T and B cells with highly substituted \(^{125}\)I-labeled FgG abrogated the adoptive FgG PFC response (specific antigen "suicide") (Table I). B-cell suicide was achieved by incubating the cells with \(^{125}\)I-labeled FgG entirely in the cold. T-cell suicide, however, required additional measures. First, the cells had to be incubated at 37°C suggesting that a metabolic process was necessary. The observation that antigen interaction with T, but not B lymphocytes, is inhibited by azide or dinitrophenol (23) is consistent with this. Second, the radioactive antigen had to be presented to the T cells on another cell surface. The different conditions required to achieve effective T- and B-cell suicide must reflect differences in the nature of the antigen-binding receptors on the two cell types. Thus, the T-cell receptor may not be sufficiently exposed on the resting cell. In order for this to take place and for antigen to be securely bound, some degree of membrane modulation may be necessary. This may best be achieved by close contact between T cell and the cell presenting antigen. There are several ways in which such an interaction could optimally occur. (a) High-affinity memory B cells might be involved. Evidence favoring this comes from our finding that antigen bound to Ig determinants on carrier-primed B cells was highly effective in activating T cells to that carrier (24). (b) An Fc receptor-bearing cell would achieve the same effect by binding antigen complexed with antibody. Indeed there is evidence for a role of B cells (25) and macrophages (26) in
enhancing T-cell activation in the presence of antibody. (c) The existence on the macrophage of a receptor for T cells (27) provides an alternative method by which macrophages, in the absence of antibody, could effectively present antigen to T cells. Since, in our experiments, the radioactive antigen was presented on anti-6 serum-treated spleen cells we cannot specify which mechanism is involved.

Further differences in the specific antigen-binding sites on T and B cells were revealed when anti-\(H-2\) sera were used in an attempt to block specific antigen suicide. Only T cells were protected from the suicide effect by pretreatment with an anti-\(H-2\) Fab reagent of appropriate specificity. This strongly suggests that \(H-2\)-associated determinants are in close proximity to the specific antigen-binding sites on T cells, not on B cells. These results add weight to the evidence already available from work in several species suggesting a relationship of major histocompatibility gene products to specific antigen-binding receptors on T cells. In the chicken, anti-B locus serum inhibited the graft-vs.-host response (28). In guinea pigs, stimulation of DNA synthesis by sensitized lymphoid cells occurred optimally when the lymphocytes and macrophages were histocompatible for at least one haplotype and was blocked by antihistocompatibility serum of appropriate specificity (29). In the mouse, anti-\(H-2\) sera effectively blocked the binding of \(^{125}\text{I}\)-labeled poly-u(Tyr, Glu)-poly-d, l-Ala--poly-L-Lys to T but not to B cells (30). Furthermore, cooperation between T and B cells was generally effective only between syngeneic or semiallogeneic cells (31). In similar interactions, a nonimmunoglobulin, antigen-specific T-cell product capable of replacing T cells was inhibited by an anti-\(H-2\) serum directed against components of the \(K\) end of the \(H-2\) complex (\(K\)- and/or \(I\)-gene products) (32). Finally, results from a variety of experimental systems indicate that interaction between sensitized T cells and antigens presented on the surface of other somatic cells (such as virus-specified antigens) occurred only when at least one set of \(H-2\) antigen specificities was shared (33, 34). \(H-2\)-gene complex-associated antigens must thus be involved in the effective operation of the T-cell receptor for antigen and therefore in determining whether or not the cell will respond in a given situation.

Summary

The relationship between \(H-2\) complex-associated determinants, Fc receptors, and specific antigen-recognition sites on T and B cells was examined by binding and functional assays. The Fc receptor was detected by radiolabeled immune complexes or aggregated human IgG. Both these reagents selectively bound to B cells, not to T cells. When spleen cells, from mice primed to several antigens, were exposed to highly substituted radioactive aggregates, their capacity to transfer both a direct and indirect plaque-forming cell response to these antigens was abrogated. Addition of B cells, but not of T cells, restored responsiveness. Complexed Ig binding to Fc receptors was prevented by pretreatment of mixed lymphoid cell populations with antisera directed against membrane components on the same cell (e.g., \(H-2\)) and on other cells (e.g., \(\theta\)). The lack of specificity of inhibition was thought to be due to the formation on cell surfaces of antigen-antibody complexes which would then attach to the Fc receptor during the incubation procedure. Specific blockade of the Fc receptor however occurred
when B cells were pretreated with the Fab fragments of anti-\(H\-2\) antibody. This was demonstrated autoradiographically and by inhibition of aggregate-induced suicide. The blocking activity of anti-\(H\-2\) Fab was removed by absorption with spleen cells from thymectomized irradiated mice but not with thymus cells of appropriate specificity. This suggested that the antibodies involved had specificity for determinants on the B-cell membrane distinct from those coded by the \(K\) or \(D\) end of the \(H\-2\) complex, and either absent from, or poorly represented on, thymus cells.

Specific antigen-induced suicide of B cells was achieved simply by incubating the cells with radioactive antigen in the cold. T-cell suicide on the other hand required that the \(^{125}\text{I}\)-labeled antigen be presented to the T cells at \(37^\circ\text{C}\) on the surface of spleen cells from antigen-primed mice. Pretreatment of T cells with the Fab fragment of anti-\(H\-2\) antibody protected them from the suicide effect. By contrast no such protection of B cells could be achieved by this procedure. In other words \(H\-2\) (\(?\ \text{Ir}\))-associated determinants may not only be in close proximity to the antigen-binding site on T cells but, in addition, may be involved in the effective operation of the receptor.

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