B-LYMPHOCYTE Fc RECEPTOR-ASSOCIATED NON-H-2 ANTIGENS ARE DETERMINED BY A SINGLE POLYMORPHIC LOCUS WHICH IS LINKED TO THE Mls LOCUS

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In recent years, considerable interest and research effort has been focused on the role of the major histocompatibility complex (MHC)\(^1\) in the immune response. Interestingly, many of the characteristics of MHC genes and their products are shared by non-MHC (background) genes and their products. In the mouse, for example, these include: (a) the ability to induce graft rejection (1); (b) the ability to stimulate lymphocyte proliferation in mixed lymphocyte culture (MLC) (2); (c) regulation of the antibody response to various antigens (3-13), which in some cases is linked to the heavy chain allotype locus (9-12) or the X-chromosome (5-7); and (d) regulation of the ability to produce (14) or accept (15) suppressor factors.

Previous work from this laboratory has shown that some non-H-2 alloantigens, like the Ia antigens determined by genes in the I region of the H-2 complex, are associated with the Fc receptors of B lymphocytes (16). Thus, antibodies against such non-H-2 alloantigens inhibited the binding of Ig complexes to B-cell Fc receptors. This inhibition was specific in that the Fc portion of the alloantibodies was not required for inhibition (16), and antibodies against other lymphocyte surface antigens did not produce similar inhibition (17). Presumably, anti-Ia antibodies and anti-non-H-2 alloantibodies inhibit the same receptors since no Fc receptors were detectable on B cells after pretreatment with either reagent (16, 17). Preliminary data suggested that the Fc receptor-associated non-H-2 antigens were determined by a single gene locus (16).

The present studies were performed to further characterize the B-lymphocyte Fc receptor-associated non-H-2 antigens and the genes which determine their expression. The data indicate: (a) the antigens are expressed primarily but not exclusively on B cells and some evidence was obtained that these antigens are not identical to Fc receptors; (b) the antigens are determined by a single non-H-2 gene locus in the three strains (A/J, B10, and CBA/J) that were tested, and the products of this single locus in at least two of the strains (B10 and CBA/J) share antigenic specificities; (c) the locus is polymorphic and determines the

\(^1\) Abbreviations used in this paper: aggregated Ig, heat-aggregated fluorescein-conjugated human Cohn fraction II immunoglobulin; BSA, bovine serum albumin; FCS, fetal calf serum; fluorescein, fluorescein isothiocyanate; \(^{3}H\)TdR, \(^{3}H\)thymidine; Ir, immune response; MEM, Eagle's MEM without L-glutamine; MHC, major histocompatibility complex; MLC, mixed lymphocyte culture; PBS, Na phosphate-(0.05 M) buffered saline; rhodamine, tetramethyl rhodamine isothiocyanate; SI, stimulation index.
expression of at least four different antigens; and (d) the locus is not linked to $H-I$ or the heavy chain allotype locus, but is linked to the $Mls$ locus.

**Materials and Methods**

**Animals.** Adult mice of strains C57BL/10Sn (B10), B10.A/SgSn, A/J, C3H/HeJ, and CBA/J were purchased from The Jackson Laboratory, Bar Harbor, Maine. C3H/HeN adult mice were obtained from the Division of Research Services of the National Institutes of Health, Bethesda, Md. F1 mice B10.A x A/J, A/J x B10.A, and C3H/HeJ x CBA/J, as well as backcross mice (B10.A x A/J)F1 x A/J, (A/J x B10.A)F1 x B10.A, and (C3H/HeJ x CBA/J)F1 x C3H/HeJ were bred in our own facilities.

**Reagents.** Fluorescein isothiocyanate (fluorescein)-conjugated rabbit anti-mouse Ig (lot nos. 7292 and 8354) was purchased from Cappel Laboratories, Inc., Downingtown, Pa. This was dialyzed against phosphate (0.05 M)-buffered saline, pH 7.2 (PBS) and used at a dilution of 1/8 in PBS. Tetramethyl rhodamine isothiocyanate (rhodamine)-conjugated goat anti-rabbit Ig was purchased from Cappel Laboratories (lot no. 7707). This was dialyzed against PBS, absorbed with Sepharose-coupled normal mouse ascites proteins, and used at a dilution of 1/8 in PBS. Fluorescein-conjugated, affinity-purified goat antibodies against mouse IgG1 and IgG2 were the kind gifts of Dr. R. Asofsky, National Institutes of Health. These were used at concentrations, respectively, of 0.5 mg/ml and 0.125 mg/ml in PBS. Fluorescein-conjugated, heat-aggregated human Cohn fraction II (aggregated Ig) was prepared as previously described (18). Before use the aggregated Ig was titered on mouse spleen cells (see below: Fluorescence) and a concentration was chosen that gave a plateau value for the percentage of positive cells (generally 1-4 mg/ml). Soluble rabbit anti-trinitrephenol (TNP)-TNP-bovine serum albumin (BSA) complexes were prepared as previously described (19) and were used at a concentration of 1 mg/ml in PBS. The affinity-purified rabbit IgG anti-TNP used in these complexes was the kind gift of Dr. P. A. Henkart, National Institutes of Health. Alloantibodies were prepared as previously described (16, 20). The alloantisera were decomplemented and used either undiluted or at dilutions which were on the plateau of cytotoxic killing for the strain of cells being tested (17). All reagents (except for rabbit anti-TNP-TNP-BSA complexes and aggregated Ig) were ultracentrifuged to remove complexes immediately before use (17). Absorptions of alloantibodies with spleen cells were performed as previously described (20).

**Fluorescence.** Single cell suspensions from spleen were prepared as previously described (17), and resuspended in 2% BSA in PBS at 20 x 10^6/ml. Na azide (0.02%) was present in all experiments except where capping was performed. The BSA-PBS had a pH of 7.2 except when it was used with aggregated Ig in which case the pH was 8.0. For detection of lymphocyte surface Ig, 25 μl of the spleen cell suspension was mixed with 25 μl of the fluorescein-conjugated rabbit anti-mouse Ig, incubated 30 min at 4°C, and washed three times with BSA-PBS. For detection of B-lymphocyte Fc receptors using aggregated Ig, 25 μl of the spleen cell suspension was mixed with 25 μl of the aggregated Ig, incubated 30 min at 23°C, and washed three times with BSA-PBS. For detection with antigen-antibody complexes, 25 μl of the spleen cell suspension was mixed with 10 μl of rabbit anti-TNP-TNP-BSA complexes and incubated 30 min at 23°C. The cells were washed three times with BSA-PBS and resuspended in 25 μl of rhodamine-conjugated goat anti-rabbit Ig. After 30 min at 4°C the cells were washed three times with BSA-PBS. For detection of lymphocyte alloantigens, 25 μl of the spleen cell suspension was incubated with 100 μl of the appropriate alloantibodies for 30 min at 4°C. The cells were washed three times with BSA-PBS and resuspended in 25 μl of fluorescein-conjugated goat anti-mouse IgG2 and, in some experiments, 25 μl of fluorescein-conjugated goat anti-mouse IgG1. After 30 min at 4°C the cells were washed three times with BSA-PBS. Preparation of slides and visual microscopy for fluorescein and rhodamine were performed as previously described (17, 18).

**Inhibition Studies.** To assess inhibition of binding of aggregated Ig to B-cell Fc receptors by the alloantibodies being tested, 25 μl of the spleen cell suspension was incubated in 100 μl of heat-inactivated alloantisemrum for 30 min at 4°C. The cells were then washed three times with BSA-PBS and labeled with aggregated Ig as described above.

**Capping of B-Lymphocyte-Bound Antigen-Antibody Complexes.** Cells were labeled with antigen-antibody complexes as described above except that no Na azide was present and the final washes were in Eagle’s MEM without L-glutamine (Microbiological Associates, Bethesda, Md.)
containing 10% (vol/vol) heat-inactivated fetal calf serum (Grand Island Biological Co., Grand Island, N.Y., lot no. 762218) (FCS). The cells were resuspended in 250 μl of MEM-FCS and incubated for 15 min in a 37°C waterbath. The cells were then washed three times with BSA-PBS containing Na azide.

**Determination of Immunoglobulin Allotype.** The anti-allotype antisera were produced by injecting anti-pertussis-pertussis complexes into inbred mice which were allotypically different as described by Herzenberg and Herzenberg (21). The Ouchterlony double diffusion technique was used to determine the allotypes of individual animals.

**Cytotoxicity.** Spleen cells were serologically typed by using a two-stage complement-mediated trypan-blue exclusion microcytotoxicity assay as previously described (22).

**Mixed Lymphocyte Reactions.** The techniques utilized have been described in detail (23, 24). In brief, 2 × 10⁶ Ficoll-Hypaque purified peripheral blood mononuclear cells as responders and 5 × 10⁵ spleen cells (treated with 2,000 rads before culture) as stimulators were mixed in 200 μl of medium in sterile tissue culture microtiter plates (no. 3040, Falcon Plastics, Division of BioQuest, Oxnard, Calif.). The medium was RPMI-1640 (Grand Island Biological Co.) containing 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 25 mM HEPES buffer, and 10% (vol/vol) heat-inactivated FCS. Cultures were labeled with [3H]thymidine ([3H]TdR) at 72 h and harvested at 90 h. Responder cells were from C3H/HeN mice or from individual (C3H/HeJ × CBA/J)F₁ × C3H/HeJ backcross mice. Stimulator cells were from (C3H/HeJ × CBA/J)F₁ mice or from individual (C3H/HeJ × CBA/J)F₁ × C3H/HeJ backcross mice. All tests were performed in triplicate. The results are calculated from the uptake of [3H]TdR and are expressed as the mean uptake of [3H]TdR in counts per minute ± SE and as the stimulation index (SI = cpm of experimental/cpm of control).

**Results**

**Analysis of B-Lymphocyte Fc Receptor-Associated Non-H-2 Antigens of the B10 and A/J Strains.** To ascertain how many loci were determining expression of non-H-2 antigens which interact with B-cell Fc receptors, (B10.A × A/J)F₁ × A/J backcross mice were evaluated for inhibition of binding of aggregated Ig complexes (which bind primarily to B-lymphocyte Fc receptors, [17]) by alloantibodies against the B10 background (Table I). Pretreatment of spleen cells with these alloantibodies caused inhibition of binding of Ig complexes (53–86%) in 14 of the 29 backcross mice analyzed. This number was not significantly different from the 50% expected if B-cell Fc receptor-associated non-H-2 antigens were determined by a single locus, but was significantly different (P < 0.01) from the 75% expected if such antigens were determined by two gene loci as evaluated by using the individual terms of the binomial distribution. Thus, B-cell Fc receptor-associated non-H-2 antigens appeared to be determined by a single locus, at least in the B10 strain. Inhibition of binding was never complete (53–86%) and the reasons for this are not yet known. However, the most likely explanation is that there are other spleen cell populations whose Fc receptors are not inhibited by these anti-non-H-2 alloantibodies. Thus, a similar lack of complete inhibition of binding of Ig complexes to spleen cells by anti-1a antibodies was observed (17). In this case, evaluation of purified cell populations has revealed that the Fc receptors of nearly all B lymphocytes are associated with 1a antigens, while those of macrophages and the effector cells in antibody-dependent cell-mediated cytotoxicity are not (25, 26). This view is further supported by the observation that pretreatment with alloantibodies against both H-2 and non-H-2 antigens also does not result in complete inhibition (data not shown). Spleen cells from the (B10.A × A/J)F₁ × A/J
**Table 1**

*Analysis of (B10.A × A/J)F1 × A/J Backcross Mice for B-Lymphocyte Fc Receptor-Associated B10 Non-H-2 Antigens, Sex, Coat Color, and Immunoglobulin Allotype*

| Animal no. | Coat color | Sex | Ig allotype | Cytotoxicity Percent killed§ | Aggregated Ig Binding | Control Exp. Percent positive | Exp. Percent positive | Inhibition % |
|------------|------------|-----|------------|-------------------------------|----------------------|-----------------------------|------------------------|-------------|
| 351        | Black      | M   | aa         | >80                           | 51.0                 | 14.5                        | 72                     |             |
| 352        | Black      | M   | aa         | >80                           | 52.0                 | 50.0                        | 4                      |             |
| 353        | Black      | M   | aa         | >80                           | 52.0                 | 9.0                         | 83                     |             |
| 355        | Black      | M   | aa         | 46                            | 52.5                 | 54.0                        | -3                     |             |
| 357        | Black      | M   | aa         | >80                           | 52.0                 | 14.0                        | 73                     |             |
| 354        | Black      | M   | ab         | 40                            | 49.0                 | 50.0                        | -2                     |             |
| 356        | Black      | M   | ab         | >80                           | 50.0                 | 12.0                        | 76                     |             |
| 370        | Black      | F   | aa         | >80                           | 39.5                 | 12.0                        | 70                     |             |
| 367        | Black      | F   | ab         | >80                           | 52.0                 | 7.5                         | 86                     |             |
| 368        | Black      | F   | ab         | >80                           | 40.5                 | 12.0                        | 70                     |             |
| 369        | Black      | F   | ab         | >80                           | 53.5                 | 16.5                        | 69                     |             |
| 362        | Black      | M   | aa         | NT                            | 51.0                 | 10.0                        | 61                     |             |
| 363        | White      | M   | aa         | >80                           | 47.0                 | 46.5                        | 1                      |             |
| 364        | White      | M   | aa         | 15                            | 42.0                 | 42.5                        | -1                     |             |
| 365        | White      | M   | aa         | 68                            | 41.5                 | 43.5                        | -5                     |             |
| 366        | White      | F   | aa         | 78                            | 46.0                 | 47.0                        | -2                     |             |
| 367        | White      | F   | ab         | NT                            | 39.5                 | 40.5                        | -3                     |             |
| 368        | White      | F   | ab         | NT                            | 40.0                 | 44.0                        | -10                    |             |
| 369        | White      | F   | ab         | NT                            | 44.0                 | 10.0                        | 77                     |             |
| 364        | White      | M   | aa         | >80                           | 44.5                 | 45.0                        | -1                     |             |
| 365        | Brown      | M   | aa         | >80                           | 48.0                 | 45.0                        | 6                      |             |
| 366        | Brown      | M   | ab         | >80                           | 47.0                 | 12.0                        | 74                     |             |
| 361        | Brown      | M   | ab         | >80                           | 40.0                 | 11.5                        | 71                     |             |
| 372        | Brown      | F   | aa         | >80                           | 40.5                 | 39.0                        | 4                      |             |
| 371        | Brown      | F   | ab         | >80                           | 40.5                 | 44.0                        | -9                     |             |
| 373        | Brown      | F   | ab         | 79                            | 40.5                 | 19.0                        | 53                     |             |

* Antibodies used were A/J anti-B10 except in animals 352 and 362 where A/J anti-B10.A antibodies were used.

‡ The percent killed shown is the highest percentage killed at any antibody concentration. Complement and serum controls were 10% killed except for animals 368, 371, 372, and 373 where the complement controls showed 12, 15, 15, and 15% killed, respectively.

|| Percent inhibition = (Percent positive, control − percent positive, experimental) × 100

Experimental = pretreatment with alloantibodies listed in footnote *. Control = pretreatment with nonreactive alloantibodies (B10.A anti-B10).

† NT = not tested.

‡ a = IgG; b = IgM.
backcross mice were also analyzed for reactivity with the anti-B10 non-H-2 alloantibodies by the microcytotoxicity assay (Table I). It was apparent that these antibodies detected multiple antigens determined by a number of different loci since there was at least partial killing of the spleen cells in 23 of the 24 mice tested. Moreover, in a number of animals (352, 358, 359, 362, 371, and 372) virtually all the cells were killed in the cytotoxic assay, yet no inhibition was seen in the aggregated Ig binding assay. This indicated the presence of non-H-2 antigens on B cells which did not interact with Fc receptors. Thus, association with B-lymphocyte Fc receptors was a characteristic of some non-H-2 antigens present on the B cell but not others.

These same backcross mice were also evaluated for sex, coat color, and immunoglobulin allotype to determine if the locus which determines expression of B-cell Fc receptor-associated non-H-2 antigens (as determined by inhibition of aggregated Ig binding) was linked to either H-1 or the heavy chain allotype locus and if sex affected expression (Table I). B-cell Fc receptor-associated non-H-2 antigens were present in 11 of 18 pigmented mice (black or brown) and 3 of 11 albino mice; 8 of 12 mice which inherited Ig allotype from the B10 and 6 of 17 which did not; and in 7 of 16 males and 7 of 13 females. Since none of these percentages were significantly different from 50% as analyzed with the individual terms of the binomial distribution, it was concluded that the locus which determines expression of B-cell Fc receptor-associated non-H-2 antigens was not linked to H-1 nor to the heavy chain allotype locus, and expression of these antigens was neither sex-limited nor an X-linked recessive trait.

To determine if B-cell Fc receptor-associated non-H-2 antigens were present on another background (A/J) (i.e., that such antigens were not unique to the B10) and also determined by a single gene locus, spleen cells from (A/J × B10.A)F1 × B10.A backcross mice were similarly evaluated for inhibition of aggregated Ig binding by pretreatment with anti-A/J non-H-2 alloantibodies (Table II). Inhibition (69-78%) was observed in 7 of 15 backcross mice. This number was not significantly different from 50% but was significantly (P < 0.02) different from 75%. Thus, B-cell Fc receptor-associated non-H-2 antigens are present and determined by a single background locus in a second strain (A/J). It should also be noted that the Fc receptors of B lymphocytes from backcross mice which were not inhibited by anti-A/J non-H-2 alloantibodies could be inhibited by pretreatment with appropriated alloantibodies (anti-H-2 which contains anti-Ia). Thus, there was no apparent difference in the B-cell Fc receptors themselves. Additionally, since the alloantibodies used in these two backcross experiments (Table I and II) were made in strains with reciprocal backgrounds (A/J anti-B10 and B10.A anti-A/J) the results suggest, but do not prove, that the B-cell Fc receptor-associated non-H-2 antigens of the B10 and A/J are alleles.

Thus, analysis of lymphocyte surface antigens determined by background genes of the B10 and A/J strains had shown that some non-H-2 antigens but not others were associated with B-cell Fc receptors and that these antigens were determined by a single locus in each of these two strains. However, linkage of this locus to another known locus had not been established. It seemed possible that there might be linkage between the Mls locus (which
### Table II

**Analysis of \((A/J \times B10.A)F_1 \times B10.A\) Backcross Mice for B-Lymphocyte Fc Receptor-Associated A/J Non-H-2 Antigens**

| Animal no. | Aggregated Ig binding after pretreatment with:* |
|------------|-----------------------------------------------|
|            | Control | Anti-H-2 | Anti-non-H-2 |
|            | Percent positive | Percent positive | Percent inhibition | Percent positive | Percent inhibition |
| 596        | 44.0    | 13.0     | 70           | 44.0             | -1                |
| 597        | 50.0    | 12.0     | 76           | 48.5             | 3                 |
| 598        | 49.0    | 10.0     | 80           | 45.0             | 8                 |
| 599        | 48.5    | 11.5     | 76           | 14.0             | 71                |
| 600        | 47.0    | 12.0     | 74           | 14.5             | 69                |
| 651        | 47.0    | 11.5     | 76           | 10.5             | 78                |
| 652        | 49.5    | 11.5     | 77           | 11.0             | 78                |
| 653        | 46.5    | 8.5      | 82           | 15.0             | 78                |
| 654        | 39.0    | 14.0     | 64           | 39.5             | 1                 |
| 655        | 37.0    | 9.0      | 76           | 36.0             | 3                 |
| 901        | 47.0    | 14.5     | 69           | 12.0             | 74                |
| 902        | 42.0    | 4.5      | 88           | 12.5             | 70                |
| 903        | 42.0    | 8.0      | 81           | 12.5             | 70                |
| 904        | 48.0    | 6.0      | 88           | 47.0             | 2                 |
| 905        | 44.0    | 8.5      | 81           | 43.5             | 1                 |

* Antibodies used were: control = normal B10.A; anti-H-2 = B10 anti-B10.A; anti-non-H-2 = B10.A anti-A/J. Percent inhibition calculated as described in footnote 1, Table I.

Determines the expression of antigens which stimulate in MLC) and the locus which determines expression of B-cell Fc receptor-associated non-H-2 antigens, since both of these properties are also characteristics of the Ia antigens determined by genes in the I region of the \(H-2\) complex. To evaluate this possibility, the non-H-2 antigens of yet another strain, the CBA/J, were analyzed.

**Analysis of B-Lymphocyte Fc Receptor-Associated Non-H-2 Antigens of the CBA/J.** While these studies were in progress, we became aware of the studies of Tonkonogy and Winn (27), which described detection of CBA/J non-H-2 antigens (by using a C3H/HeJ anti-CBA/J alloantiserum) which were primarily expressed on B cells and peritoneal phagocytic cells, and which were determined by a single locus which was linked to the \(Mls\) locus. It seemed possible that the non-H-2 antigens which interact with B-cell Fc receptors and those that they described were the same. Therefore, C3H/HeJ anti-CBA/J alloantibodies were prepared and evaluated for binding to CBA/J spleen cells and for the ability to inhibit binding of aggregated Ig (Table III). Antigens detected by these alloantibodies were present on 53.0% of the spleen cells as evaluated by indirect fluorescence by using anti-IgG2 (which does not detect surface Ig). This was a slightly higher percentage than the percentage of Ig-bearing B cells (46.0%). Indirect fluorescence with anti-Ig after pretreatment with the anti-non-H-2 alloantibodies showed 62.5% positive cells. Further, indirect fluorescence using anti-Ig after pretreatment with both anti-Thy 1.2 and these anti-
C3H/HeJ anti-CBA/J alloantibodies bind to CBA/J B lymphocytes and inhibit binding of Ig complexes to B-lymphocyte Fc receptors

| Reagent                  | Specificity | Anti-Ig staining % | Anti-IgG2 staining % | Aggregated Ig binding % |
|--------------------------|-------------|--------------------|----------------------|-------------------------|
| Medium                   | --          | 46.0               | 0.5                  | 46.0                    |
| Normal C3H               |             | 47.0               | 1.0                  | 45.5                    |
| C3H/HeJ anti-CBA/J       | Non-H-2     | 62.5               | 53.0                 | 9.0                     |
| B10 anti-B10.BR          | H-2         | 89.0               | 97.5                 | 14.0                    |

Pretreatment of CBA/J spleen cells with these alloantibodies markedly inhibited the binding of aggregated Ig (80%). Thus, these alloantibodies detected non-H-2 antigens which were associated with B-cell Fc receptors. This observation, together with the previous observations noted above (27), suggested that the locus which determines the expression of B-lymphocyte Fc receptor-associated non-H-2 antigens was linked to the Mls locus. To confirm this, spleen cells from (C3H/HeJ x CBA/J)F1 x C3H/HeJ backcross mice were analyzed for the presence of CBA/J Mls antigens, and B-lymphocyte Fc receptor-associated non-H-2 antigens (Table IV). Of the 29 animals assessed, 13 were negative for both while 14 were positive for both Fc receptor inhibition by anti-CBA/J non-H-2 alloantibodies (48-64% inhibition) and stimulation of C3H/HeN lymphocytes in MLC (SI of 4.7 to 12.9 except for mouse M10 which had a SI of 2.3. MLC experiments between M10 and other backcross mice confirmed that this mouse did express CBA/J Mls antigens [data not shown]). Two backcross mice (M13 and M16) were positive for CBA/J Mls antigens but negative for B-cell Fc receptor-associated non-H-2 antigens. Thus, CBA/J B-cell Fc receptor-associated non-H-2 antigens were determined by a single gene locus which was linked but not identical to the Mls locus (apparent recombination frequency 6.8%).

Spleen cells from these same backcross mice were also analyzed for reactivity with the anti-CBA/J non-H-2 alloantibodies by microcytotoxicity (Table IV). In contrast to the A/J anti-B10 non-H-2 alloantibodies (see above), killing of spleen cells by the C3H/HeJ anti-CBA/J alloantibodies was only observed with cells from the same 14 backcross mice whose B-cell Fc receptors were inhibited (30-57% net kill). Thus, the only antigens detected by these alloantibodies were associated with B-cell Fc receptors or were determined by the same locus. This observation, together with the fact that these antigens were expressed primarily on B cells (see above), made it possible to evaluate whether the B-cell Fc receptor-associated non-H-2 antigens might be Fc receptors. Therefore, the distribution of these antigens was evaluated by indirect immunofluorescence before and after capping of B-lymphocyte-bound antigen-antibody complexes (Table V). Although the Ig complexes were capped on virtually all the
TABLE IV
Analysis of (C3H/HeJ × CBA/J)F1 × C3H/HeJ Backcross Mice for B-Lymphocyte Fc Receptor-Associated CBA/J-Non-H-2 Antigens, Sex, and CBA/J Mls Antigens

| Animal | MLC response of C3H/HeN Mean uptake of [3H]Tdr | Cytotoxicity, percent killed; Aggregated Ig Binding |
|--------|-----------------------------------------------|---------------------------------------------------|
|        | C3H/HeN Exp. C3H/HeN SI C' Only Exp. Nat | Control Exp. Percent positive Percent positive Percent inhibition |
|        | cpm ± SE | | | | | |
| None   | 3,257 ± 108 | 5 | 44 | 39 | 50.0 | 18.0 | 84 |
| F      | 29,119 ± 1,236 (8.6) | 11 | 12 | 1 | 52.0 | 48.0 | 8 |
| F 1    | 3,954 ± 156 (1.2) | 7 | 15 | 8 | 51.5 | 49.0 | 5 |
| F 2    | 3,759 ± 222 (1.1) | 11 | 16 | 5 | 48.0 | 45.0 | 6 |
| F 3    | 4,137 ± 114 (1.3) | 11 | 12 | 1 | 54.0 | 52.5 | 3 |
| None   | 4,251 ± 217 | | | | | | |
| F      | 41,416 ± 1,962 (9.7) | 9 | 52 | 43 | 58.0 | 24.0 | 59 |
| F 6    | 3,818 ± 273 (0.9) | 12 | 14 | 2 | 52.0 | 50.0 | 8 |
| F 7    | 4,039 ± 195 (0.9) | 4 | 12 | 8 | 57.0 | 58.0 | 2 |
| F 8    | 4,453 ± 258 (1.1) | 11 | 16 | 5 | 54.5 | 52.0 | 5 |
| F 9    | 32,416 ± 1,312 (1.7) | 10 | 14 | 4 | 54.5 | 57.0 | 9 |
| F 10   | 54,732 ± 2,413 (12.9) | 14 | 58 | 44 | 52.5 | 21.0 | 60 |
| None   | 3,346 ± 152 | 11 | 53 | 42 | 50.0 | 26.0 | 48 |
| F      | 35,364 ± 317 (10.6) | 11 | 53 | 42 | 50.0 | 26.0 | 48 |
| F 11   | 4,249 ± 232 (1.3) | 9 | 17 | 8 | 50.5 | 48.5 | 6 |
| M      | 37,634 ± 1,852 (11.2) | 12 | 50 | 44 | 49.5 | 22.5 | 55 |
| M 12   | 41,418 ± 2,019 (12.4) | 17 | 20 | 3 | 45.5 | 41.0 | 4 |
| M 13   | 36,691 ± 1,567 (10.9) | 16 | 64 | 48 | 46.0 | 24.0 | 48 |
| M 14   | 5,307 ± 268 (1.6) | 11 | 12 | 1 | 57.0 | 54.0 | 5 |
| M 15   | 28,876 ± 1,195 (8.6) | 16 | 19 | 3 | 50.0 | 48.5 | 5 |
| M 16   | 4,682 ± 124 (1.5) | 16 | 18 | 2 | 43.0 | 42.0 | 2 |
| M 17   | 5,019 ± 215 (1.5) | 18 | 23 | 5 | 56.0 | 40.0 | 5 |
| M 18   | 30,665 ± 236 (9.0) | 21 | 70 | 54 | 42.5 | 21.0 | 51 |
| None   | 3,757 ± 154 | 11 | 49 | 36 | 39.5 | 18.0 | 54 |
| F      | 28,876 ± 1,195 (10.7) | 16 | 64 | 48 | 46.0 | 24.0 | 48 |
| F 2    | 29,876 ± 1,195 (10.7) | 16 | 64 | 48 | 46.0 | 24.0 | 48 |
| M 3    | 28,657 ± 2,168 (7.7) | 7 | 56 | 49 | 40.0 | 24.0 | 59 |
| M 4    | 5,357 ± 312 (1.7) | 11 | 12 | 1 | 57.0 | 54.0 | 5 |
| M 5    | 28,876 ± 1,195 (8.6) | 16 | 19 | 3 | 50.0 | 48.5 | 5 |
| M 6    | 4,682 ± 124 (1.5) | 16 | 18 | 2 | 43.0 | 42.0 | 2 |
| None   | 5,019 ± 215 (1.5) | 18 | 23 | 5 | 56.0 | 40.0 | 5 |
| F      | 30,665 ± 236 (9.0) | 21 | 70 | 54 | 42.5 | 21.0 | 51 |
| F 10   | 9,714 ± 413 (2.3) | 15 | 51 | 36 | 39.5 | 20.0 | 49 |

* Antibodies used were: control = normal C3H/HeJ; experimental = C3H/HeJ anti-CBA/J.
‡ The percent killed shown is the highest percentage killed at any antibody concentration.
§ Percent inhibition calculated as described in footnote n, Table 1.

| Cells which bore them, the CBA/J non-H-2 antigens remained unaffected on most cells. Thus, at least some of these B-cell Fc receptor-associated non-H-2 antigens are not identical to Fc receptors.

The Locus Which Determines Expression of B-Lymphocyte Fc Receptor-Associated Non-H-2 Antigens May be Identical in the B10 and CBA/J Strains. Preliminary strain distribution studies indicated that anti-CBA/J non-H-2 alloantibodies inhibited the binding of aggregated Ig to spleen cells from B10.A but not A/J, and that anti-B10 non-H-2 alloantibodies inhibited the Fc receptors
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Table V
Capping of B Lymphocyte-Bound Ag-Ab Complexes Fails to Redistribute CBA/J Non-H-2 B-Lymphocyte Fc Receptor-Associated Antigens

| Incubation no. | Rhodamine goat anti-rabbit Ig | Fluorescein goat anti-mouse IgG, and IgG2 |
|---------------|-------------------------------|------------------------------------------|
| 1             | 25                            | 0                                        |
| 2             | 3                             | 4                                        |
| 3             | 4                             | 61.0                                     |
| 4             | 75                            | 55.0                                     |

* Spleen cells from CBA/J were used. The percentage of surface Ig-positive cells was 51.0.
† After incubation no. 2 the cells were incubated at 37°C for 15 min to induce capping. Capping was terminated by washing with 4°C medium containing NaN3 (0.02%). Incubations no. 3 and 4 were in the presence of NaN3 to prevent capping.
§ Cells were considered capped if fluorescence was detected on less than 50% of the cell surface and was at one pole of the cell. Only fluorescence-positive cells were considered in determining the percent capped.

of CBA/J but not C3H/HeJ (data not shown). Thus, it was possible to determine if B-lymphocyte Fc receptor-associated non-H-2 antigens might be determined by the same gene locus in these two strains. Spleen cells from (B10.A x A/J)F1 x A/J and (C3H/HeJ x CBA/J)F1 x C3H/HeJ backcross mice were evaluated for inhibition of aggregated Ig binding by pretreatment with anti-B10 non-H-2 alloantibodies and anti-CBA/J non-H-2 alloantibodies (Tables VI and VII). There was a perfect correlation of Fc receptor inhibition by these two reagents in both groups of backcross mice, i.e., the B-lymphocyte Fc receptors of an individual mouse were either inhibited by both anti-B10 non-H-2 alloantibodies and anti-CBA/J non-H-2 alloantibodies or by neither. This suggested that the locus which determined expression of B-cell Fc receptor-associated non-H-2 antigens was probably identical in the B10 and CBA/J strains.

To ascertain if this locus determined B-cell Fc receptor-associated antigen(s) which were not shared or crossreactive between the B10 and CBA/J, as well as antigen(s) that were, absorption studies were performed. Anti-B10 non-H-2 alloantibodies were absorbed with CBA/J spleen cells until no longer reactive as evaluated by microcytotoxicity (2 x 10⁹ cells/ml), and anti-CBA/J non-H-2 alloantibodies were absorbed with B10 spleen cells until no longer reactive (1.6 x 10⁹ cells/ml). The absorbed alloantibodies were then evaluated for their ability to inhibit binding of aggregated Ig to spleen cells from each of these strains (Table VIII). Anti-B10 non-H-2 alloantibodies absorbed with spleen cells from CBA/J mice no longer inhibited the B-cell Fc receptors of spleen cells from CBA/J mice but continued to inhibit those of spleen cells from B10 mice. Similarly, anti-CBA/J non-H-2 alloantibodies absorbed with spleen cells from B10 mice did not inhibit binding of Ig complexes to B10 spleen cells but did inhibit such binding by CBA/J spleen cells. Thus, each of these two strains had at least one antigen which was not shared or crossreactive with the other, and at least one antigen which was shared. Moreover, each of the three antigens so far detected was associated with B-lymphocyte Fc receptors.

Discussion
The specificity of inhibition of the binding of heat-aggregated Ig complexes
Analysis of \((B10.A \times A/J)F_1 \times A/J\) Backcross Mice for Inhibition of B-Lymphocyte Fc Receptors by Alloantibodies against \(B10\) and CBA\(/J\) Non-H-2 Antigens

| Animal no. | Control | Percent positive | Percent positive | Percent inhibition | Percent positive | Percent inhibition |
|------------|---------|-----------------|-----------------|-------------------|-----------------|-------------------|
| 101        | 32.0    | 30.0            | 30.5            | 5                 |
| 102        | 32.5    | 32.0            | 34.0            | -5                |
| 103        | 36.0    | 12.0            | 16.0            | 56                |
| 104        | 44.0    | 41.0            | 44.5            | -1                |
| 106        | 49.0    | 19.0            | 18.0            | 63                |
| 107        | 49.0    | 50.0            | 48.0            | 2                 |
| 108        | 38.0    | 35.5            | 36.0            | 5                 |
| 109        | 56.5    | 56.0            | 52.0            | 8                 |
| 201        | 49.0    | 46.0            | 50.0            | -2                |
| 202        | 44.0    | 10.5            | 14.0            | 68                |
| 203        | 52.0    | 6.0             | 10.0            | 61                |
| 204        | 53.5    | 46.0            | 51.0            | 3                 |
| 205        | 59.5    | 61.0            | 62.0            | -4                |
| 207        | 47.0    | 8.0             | 17.0            | 64                |
| 208        | 43.0    | 42.0            | 41.5            | 3                 |
| 209        | 42.0    | 42.5            | 41.0            | 2                 |
| 210        | 38.0    | 4.5             | 11.5            | 70                |
| 211        | 49.5    | 50.0            | 47.0            | 5                 |

* Antibodies used were: control = normal C3H/HeJ; anti-B10 non-H-2 = A/J anti-B10; anti-CBA/\(J\) non-H-2 = C3H/HeJ anti-CBA/J. Percent inhibition calculated as described in footnote I, Table I.

To the Fc receptors of B lymphocytes by alloantibodies against Ia antigens and some non-H-2 alloantigens has been previously established (28). The present results indicate that this is a characteristic of antibodies against certain non-H-2 alloantigens but not others (including some that are expressed on B cells, see Table I). The B-lymphocyte Fc receptor-associated non-H-2 antigens are determined by the gene(s) of a single locus in the three strains that have been analyzed (B10, A/J, and CBA/J, see Tables I, II, and IV). Moreover, the observation that at least one such antigen is shared or crossreactive between B10 and CBA/J and is present in the same backcross mice which also had noncrossreactive antigens suggests that the locus which determines expression of B-lymphocyte Fc receptor-associated non-H-2 antigens is identical in these two strains (Tables VI and VII). It seems likely that an identical locus will be present in all mouse strains. At least four different antigens (one each in A/J, B10, and CBA/J and one which is shared or crossreactive between B10 and CBA/J, see Tables II and VIII) have been identified each of which is associated with B-cell Fc receptors. This locus is thus polymorphic and may in fact be a gene complex. Studies involving strain distribution, absorptions, and chemical characterization will be necessary to further define the number of antigens involved and clarify this point. The antigens involved are expressed primarily on B lymphocytes, at least in CBA/J mice (Table III and reference 27).
### Table VII

**Analysis of (C3H/HeJ × CBA/J)F1 × C3H/HeJ Backcross Mice for Inhibition of B-Lymphocyte Fc Receptors by Alloantibodies against B10 and CBA/J Non-H-2 Antigens**

| Animal no. | Control Aggregated Ig binding after pretreatment with:* | Anti-CBA/J non-H-2 | Anti-B10 non-H-2 |
|------------|---------------------------------------------------------|--------------------|-----------------|
|            | Percent positive | Percent positive | Percent inhibition | Percent positive | Percent inhibition |
| 1          | 49.0            | 23.0             | 53               | 24.5            | 50               |
| 2          | 39.5            | 18.0             | 54               | 19.0            | 52               |
| 3          | 48.0            | 24.0             | 50               | 20.0            | 58               |
| 4          | 47.5            | 48.0             | 1                | 47.0            | 1                |
| 5          | 53.5            | 23.0             | 57               | 18.0            | 86               |
| 6          | 49.0            | 25.0             | 49               | 26.0            | 47               |
| 7          | 56.0            | 20.0             | 64               | 19.5            | 65               |
| 8          | 46.0            | 18.0             | 61               | 20.0            | 57               |
| 9          | 42.0            | 18.0             | 57               | 21.0            | 50               |
| 10         | 39.5            | 20.0             | 49               | 19.0            | 52               |
| 11         | 50.5            | 48.5             | 6                | 52.0            | 5                |
| 12         | 49.5            | 22.5             | 55               | 24.0            | 52               |
| 13         | 42.5            | 41.0             | 4                | 38.0            | 11               |
| 14         | 46.0            | 24.0             | 48               | 22.0            | 52               |
| 15         | 57.0            | 54.0             | 5                | 58.0            | 2                |
| 16         | 49.0            | 48.5             | 1                | 50.0            | 2                |
| 17         | 43.0            | 42.0             | 2                | 45.0            | 5                |
| 18         | 38.0            | 40.0             | -5               | 42.5            | -12              |
| 19         | 42.5            | 21.0             | 51               | 23.0            | 46               |

* Antibodies used were: control = normal C3H/HeJ; anti-CBA/J non-H-2 = C3H/HeJ anti-CBA/J; anti-B10 non-H-2 = A/J anti-B10.A; percent inhibition calculated as described in footnote U, Table I.

### Table VIII

**Several (at Least Three) B-Lymphocyte Fc Receptor-Associated Non-H-2 Antigens are Determined by the Single Locus Apparently Common to the B10 and CBA/J Genetic Backgrounds**

| Exp. no. | Alloantibodies | Aggregated Ig binding |
|----------|----------------|-----------------------|
|          |                | B10  | CBA/J  |
|          |                | Percent positive     | Percent positive |
| 1        | Normal C3H     | 52.0 | 50.5   |
|          | C3H/HeJ anti-CBA/J | 9.0 | 10.5   |
|          | C3H/HeJ anti-CBA/J absorbed with B10 | 51.5 | 17.0 |
| 2        | Normal A/J     | 50.5 | 52.0   |
|          | A/J anti-B10.A | 7.0  | 14.0   |
|          | A/J anti-B10.A absorbed with CBA/J | 19.0 | 50.5 |

Absorption studies will be necessary to determine if this is also true in the other strains. It is of interest that in one case (C3H/HeJ anti-CBA/J tested on the CBA/J background) the anti-non-H-2 alloantibodies were specific for antigens which
were B-lymphocyte Fc receptor-associated, while in another case (A/J anti-B10 tested on the B10 background) the anti-non-H-2 alloantibodies also detected other non-H-2 antigens (including some which were present on B lymphocytes). In the latter case, it should be possible to obtain alloantibodies specific for B10 B-lymphocyte-associated non-H-2 antigens by absorbing with lymphocytes from backcross mice who did not inherit such antigens (see Table I). Alternatively, one could breed such mice and use the cells of their progeny. In this fashion, it is theoretically possible to obtain alloantibodies specific for B-lymphocyte Fc receptor-associated non-H-2 antigens in any strain.

Capping experiments (Table V) revealed that at least some of the non-H-2 antigens which are associated with B-cell Fc receptors are not themselves Fc receptors. Since it seems likely that multiple antigens are involved, it remains possible that some but not others are Fc receptors. Monospecific alloantibodies will be required to clarify this point. However, these results are consistent with the possibility that the association is similar to the association between La antigens and B-cell Fc receptors. Recent distributional and quantitative studies have indicated that the latter is probably an interaction induced by the binding of ligands to La antigens (29). It has been hypothesized that this association may play a role in regulation of the immune response (29, 30).

Linkage studies revealed that the locus which determines expression of B-cell Fc receptor-associated non-H-2 antigens was not linked to H-1 or the heavy chain allotype locus and was neither sex limited nor an X-linked recessive trait (Table I). However, there was close linkage to the Mls locus (Table IV). This locus and the Mls locus are probably not identical since recombination was seen in 2 of 29 animals. Since Tonkonogy and Winn did not observe recombination in 42 animals (27), further analysis of backcross mice as well as progeny testing of putative recombinant animals will be required to determine the exact frequency of recombination. Additional evidence that these loci are not identical comes from the observation that the B10 and CBA/J share one B-cell Fc receptor-associated antigen and yet the B10 Mls locus is nonstimulating (2). Finally, recent strain distribution studies also suggest that these two loci are not identical.2

There are a variety of similarities between the non-H-2 locus characterized in this paper and the H-2-linked I region. Both are polymorphic gene systems, the products of which are expressed predominantly but not exclusively on the B-cell subpopulation of lymphoid cells. The products of both are associated specifically with the B-cell Fc receptor, and both are closely linked if not identical to genes determining MLC stimulatory antigens. In the case of La antigens, several studies have implied that these antigens are identical to the MLC stimulatory antigens (31, 32), although close linkage genetically and close association on the cell surface could also explain the data. On the other hand, our backcross data, as well as strain distribution, point to close linkage but separability of the two comparable non-H-2 loci. By analogy to the studies of MLC stimulation by La antigens, it will be of interest to determine whether

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2 S. L. Tonkonogy and H. J. Winn. 1977. Further genetic and serologic analysis of the Lym-1 alloantigenic system. *Immunogenetics.* 5:57.
or not antibodies to the non-H-2 Mls-associated antigens are capable of blocking Mls stimulations.

This extensive similarity of properties suggests that the Mls locus might better be termed the Mls complex, by analogy to the MHC. Theoretically, homology with the I region of the MHC might imply evolution of this system via duplication and translocation of an early (? primordial) I region gene. If so, one might expect structural homologies to be found between the products of the Mls complex and the 1a antigens, something which should be experimentally testable. If structural homology were indeed found, it would be important to determine at what point separation of the two chromosomal regions occurred during evolution, and whether similar non-MHC-linked complexes exist in other species. For example, if such loci exist in man, they could be of importance in tissue typing. Such typing might provide an important adjunct to HLA typing for transplants, especially of bone marrow in which GVH reactions are often seen even after HLA identical grafts.

Finally, the definition of the murine I region stemmed originally from the detection of immune response (Ir) genes determining responsiveness to a variety of immunogens. By analogy, one might predict that the Mls complex may also be involved in Ir gene function. While the majority of Ir genes studied to date have been H-2 linked, there have been several responses described, the linkage relationships of which have not been determined (3, 4, 8, 13). Attempts to examine possible linkage between such responses and the Mls complex are currently in progress.

Summary

Certain non-H-2 alloantigens are associated with murine B-lymphocyte Fc receptors in that pretreatment of spleen cells with alloantibodies against these antigens inhibited binding of Ig complexes to B-cell Fc receptors. This inhibition was specific in that: (a) as has been shown previously, the Fc portion of the alloantibody was not required to produce inhibition; and (b) antibodies against some non-H-2 antigens but not antibodies against others (including some that were expressed on B cells) caused such inhibition.

Backcross experiments revealed that the B-cell Fc receptor-associated non-H-2 antigens were determined by the gene(s) of a single background locus in each of the three strains tested (A/J, B10, and CBA/J). This locus was polymorphic in that at least four different B-cell Fc receptor-associated non-H-2 antigens were identified (one each in the A/J, B10, and CBA/J and one antigen shared or crossreactive between the B10 and CBA/J). These antigens were primarily but not exclusively expressed on B lymphocytes as determined by immunofluorescence studies, and on the basis of capping experiments they did not appear to be identical to B-cell Fc receptors.

Linkage studies revealed that the locus which determined these antigens was not linked to the albino locus nor the heavy chain allotype locus and expression was neither sex-limited nor an X-linked recessive trait. However, this locus was closely linked but not identical to the Mls locus (apparent recombination frequency 6.8%).

Thus, two closely linked non-H-2 loci both determine the expression of
antigens which have characteristics similar to Ia antigens. One locus is polymorphic and determines the expression of antigens which are primarily expressed on B cells and are specifically associated with the Fc receptors of these cells. The other (Mls locus) determines antigens which are stimulatory in mixed lymphocyte cultures. These observations suggest that there may be a second gene complex which is the analogue of the I region of the H-2 complex.

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