I-REGION GENES ARE EXPRESSED ON T AND B LYMPHOCYTES

Studies of the Mixed Lymphocyte Reaction (MLR)*

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A small region of the major histocompatibility complex (MHC)† of both the mouse and man has been found to be associated with several important immunological phenomena (1-2). This region in the mouse is situated between genes H-2K, the K end of H-2, and Ss,Slp (a serum protein gene) within the H-2 complex, and is called the I region (3). The I region contains the immune response (Ir) genes which regulate humoral and certain aspects of cell-mediated immune responses to a number of antigens (4-7). Genes controlling strong mixed lymphocyte reactions (MLR) and graft-vs.-host reactions (GVHR), in MHC different combinations, also have been localized in the I region (8-10). Another immunological phenomenon, the interaction of T and B lymphocytes in adoptive transfer systems, also appears to be affected by genes associated with the K end of the H-2 complex (11). In addition, the I region codes for a series of lymphocyte cell surface antigens, the Ia antigens, which can be detected by alloantisera raised in congenic mice differing only within the I region (references 12-16 and footnote 2). Whether these four phenomena—Ir, MLR-GVHR, “interaction barrier,” and Ia—are experimental representations of only one or of several distinct gene products is presently unknown.

Because of methodological simplicity and reproducibility, anti-Ia sera and MLR are useful analytical tools for the study of the function and genetics of the I region. Additionally, the sera directed against Ia specificities are promising reagents for the detection of lymphocyte receptors used as inhibitors of various immune reactions controlled by I-region genes. However, the anti-Ia sera studied thus far have been found to be predominantly directed against B-cell specificities (15, 16). Since there is a considerable amount of evidence that Ir genes are expressed on T cells (1, 17), or on both T and B cells (18), we decided to perform...
some direct experiments to study the expression of T-cell antigens controlled by
the I region.

Materials and Methods

Animals. 2- to 5-mo old mice of both sexes were used. In one experiment animals of the same sex
have been used. The strains and their genetic make up are summarized in Table I.

MLR Cultures. Unidirectional MLR cultures were established in Falcon Microtest II plates
(Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) in 0.2 ml/well containing 1 x 10^8 responder and 1
x 10^6 irradiated (3,000 rad from a ^{137}Cs radiation source) stimulator cells in medium RPMI-1640
(Grand Island Biological Co., Grand Island, N. Y.) supplemented with 5% human serum, 0.002 mM
glutamine, 5 x 10^{-8} M 2-mercaptoethanol, 100 U penicillin and 100 µg streptomycin/ml, and 100
µCi [^{3}H]thymidine (New England Nuclear Co., Boston, Mass., 6.7 Ci/mol) for about 16 h. The cells
were harvested onto glass wool fiber filters in a Mash II (Microbiological Associates, Inc., Bethesda,
Md.) cell harvester, and the radioactivity was measured in a 2,5-diphenyloxazole, 1,4-bis[2-(5-
phenyloxazolyl)]benzene, and toluene scintillation mixture in a liquid scintillation spectrometer.

Preparation of Cell Suspensions. Cell suspensions were made by gentle teasing of the organs with
two pairs of sharp forceps. Thymocytes were prepared from thymuses after the removal of the
parathyrmic lymph nodes, stained previously by intraperitoneally injected india ink.

Separation of T and B lymphocytes. T and B cells were isolated from lymph node cell suspen-
sions. T cells were separated by the nylon wool filtration technique (19) or by passing the cells through
a Sepharose column coupled with polyvalent antimouse immunoglobulin. The B cells were separated
by removing the T cells with anti-Thy 1.2 serum and complement followed by the removal of dead cells
by centrifugation through a 35% bovine serum albumin gradient. The separated cells were tested in
every experiment by anti-Thy 1.2 serum titration. No T-cell preparates under 90% and no B-cell
preparations above 10% Thy 1.2 positivity were used in the experiments.

Results

The rationale of the experiments was the following: If the I region does not code
for T-cell antigens, then T cells should not stimulate the MLR across differences
in the I region alone. Conversely, a positive reaction would indicate that the I
region does control T-cell surface antigens.

Five combinations between eight mouse strains have been used in the MLR
cultures. The H-2 map of these can be seen in Table I. The combinations have
been chosen so that they should comprise a series of decreasing genetic
differences within the H-2 complex. Combination 1 (C3H and C3H.SW) differs in
the whole H-2 complex; combinations 2 and 3 [A.TL and A.TH; B10.T(6R) and
B10.AQR] differ in the whole I region, but are identical in H-2K and H-2D.
Finally, combinations 4 and 5 [(B10.HTT x A/J)F1 and A.TH; B10.S(7R) and
B10.HTT] differ only in one I subregion (I-C) and Ss,Slp, and the latter appears
to have no effect in the MLR (8). The five combinations were tested in three
different experimental sets (Tables II-IV). First, the reactions of lymph node
lymphocytes against lymph node lymphocytes and thymocytes were compared
(Table II). In the second set of experiments, lymph node lymphocytes were the
responder cells stimulated by purified lymph node T or B cells (Table III).
Finally, purified T cells as responder cells were reacted against purified T or B
stimulator cells (Table IV).

The results can be seen in Tables II-IV. Five points of interest emerge from a
review of these results: (a) In all three experimental sets, a complete MHC
Table I

Genetic Combinations Used in the MLR

| Combination | Strain | H-2 haplotype | H-2 genotype | Genetic difference |
|-------------|--------|---------------|--------------|--------------------|
|             |        | H-2K | Ir-1A | Ir-1B | I-C | Ss,Slp | H-2D |
| 1           | C3H/DiSn | k | k | k | k | k | k | Whole H-2 |
|             | C3H.SW   | b | b | b | b | b | b |
| 2           | A.TL     | t1 | s | k | k | k | k | Ir-1A, Ir-1B |
|             | A.TH     | t2 | s | s | s | s | s | I-C, Ss,Slp |
| 3           | B10.AQR  | y1 | q | k | k | d | d | Ir-1A, Ir-1B |
|             | B10.T(6R) | y2 | q | q | q | q | q | I-C, Ss,Slp |
| 4           | (B10.HTT × A/J)F1 | t1/a | s/k | s/k | s/k | k/d | k/d | I-C, Ss,Slp |
|             | A.TH     | t1/a | s | s | s | s | s | d |
| 5           | B10.HTT  | t2 | s | s | s | k | k | I-C, Ss,Slp |
|             | B10.S(7R) | t2 | s | s | s | s | s | d |

Table II

MLR against Lymph Node and Thymus Lymphocytes as Stimulator Cells

| Combination | Strains | Cells | Syngeneic | Allogeneic | Stimulation index | t test P |
|-------------|---------|-------|-----------|------------|------------------|----------|
|             | Responder | Stimulator | Responder | Stimulator | cpm ± SD | cpm ± SD | | |
| 1           | C3H/DiSn | C3H.SW | L* | L | 2,517 | 325 | 62,267 | 8,970 | 24.84 | 0.001 |
|             | C3H/DiSn | C3H.SW | L | Thy** | 955 | 132 | 6,490 | 1,330 | 6.80 | 0.001 |
| 2           | A.TL | A.TH | L | L | 7,590 | 591 | 22,569 | 5,205 | 2.97 | 0.001 |
|             | A.TL | A.TH | L | Thy | 8,123 | 3,239 | 23,454 | 3,923 | 2.89 | 0.001 |
|             | A.TL | A.TH | L | L | 3,901 | 505 | 34,385 | 12,186 | 2.95 | 0.001 |
|             | A.TL | A.TH | L | Thy | 2,630 | 182 | 9,947 | 1,029 | 3.78 | 0.001 |
| 3           | B10.T(6R) | B10.AQR | L | L | 9,184 | 3,430 | 39,208 | 1,746 | 4.72 | 0.001 |
|             | B10.T(6R) | B10.AQR | L | Thy | 6,957 | 4,546 | 38,877 | 4,504 | 5.59 | 0.001 |
|             | B10.AQR | B10.T(6R) | L | L | 8,410 | 2,214 | 19,209 | 3,058 | 2.28 | 0.001 |
|             | B10.AQR | B10.T(6R) | L | Thy | 6,957 | 4,546 | 38,877 | 4,504 | 5.59 | 0.001 |
| 4           | (B10.HTT × A/J)F1 | A.TH | L | L | 924 | 242 | 1,274 | 156 | 1.38 | 0.05 |
|             | (B10.HTT × A/J)F1 | A.TH | L | Thy | 629 | 117 | 5,099 | 899 | 8.11 | 0.001 |

* L, lymph node lymphocyte.
† Thy, thymocyte.

Difference (C3H versus C3H.SW) gave a stronger reaction than the I-region differences, in agreement with previous findings of others (8), and our own unpublished results. (b) T lymphocytes (thymocytes and lymph node T cells) did stimulate in all of the combinations. (c) Lymph node T cells stimulated as well or better in the MLR than thymocytes. This finding rules out the possibility that the stimulation generated by thymocytes was due to a Tla-antigen difference.
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### TABLE III

**MLR against Purified Lymph Node T and B Cells**

| Combination | Strains | Cells | Syngeneic | Allogeneic | Stimulation index | t test | P < 10-2 |
|-------------|---------|-------|-----------|------------|-------------------|--------|---------|
|             | Responder | Stimulator | cpm ± SD | cpm ± SD |                  |        |         |
| 1           | C3H/DiSn | C3H.SW | L* T↓ | 20,876 ± 2,915 | 110,416 ± 16,408 | 5.29 | 0.001 |
|             | C3H/DiSn | C3H.SW | L* T↓ | 8,388 ± 3,238 | 120,554 ± 4,268 | 14.37 | 0.001 |
|             | C3H.SW   | C3H   | L T  | 10,624 ± 2,980 | 192,574 ± 10,527 | 9.65  | 0.001 |
|             | C3H.SW   | C3H   | L B  | 3,912 ± 690   | 145,623 ± 4,075  | 37.22 | 0.001 |
| 2           | A.TL     | A.TH  | L T  | 24,714 ± 2,976 | 122,205 ± 23,933 | 4.95  | 0.001 |
|             | A.TL     | A.TH  | L B  | 13,200 ± 3,251 | 44,308 ± 6,544  | 3.36  | 0.001 |
| 3           | B10.AQR  | B10.T(6R) | L T  | 2,017 ± 464   | 5,134 ± 1,580  | 2.55  | 0.01  |
|             | B10.AQR  | B10.T(6R) | L B  | 2,120 ± 383   | 5,922 ± 297   | 2.80  | 0.001 |
| 4           | (B10.HTT × A/J)F → | A.TH | L T  | 2,924 ± 503   | 15,563 ± 978  | 4.64  | 0.001 |
|             | (B10.HTT × A/J)F → | A.TH | L B  | 1,868 ± 754   | 2,161 ± 43    | 1.20  | NS     |
| 5           | B10.S(7R) | B10.HTT | L T  | 5,416 ± 1,747 | 12,051 ± 1,034 | 2.25  | 0.01  |
|             | B10.S(7R) | B10.HTT | L B  | 18,334 ± 4,476 | 17,644 ± 2,853 | 0.97  | NS     |

* L, lymph node lymphocyte.
† T, nylon wool purified lymph node T cells.
‡ B, anti-Ω + C' purified lymph node B cells.
§ NS, not significant.

The Tla gene is located close to H-2D and is expressed only in thymocytes of normal animals (20). Therefore, the stimulation generated by lymph node T cells could not be due to the Tla antigens. (d) Isolated lymph node T cells, as responder cells, gave essentially the same pattern of response to T- and B-cell stimulators as did lymph node cell suspensions (Table IV), suggesting that the reaction against T-stimulator cells was not the result of "back stimulation." In the case of back stimulation, the irradiated T-stimulator cells would presumably have responded towards the nonirradiated B cells in the responder lymph node cell suspension by secreting some mitogenic factor, thus triggering the responder cells to multiplication (21-22). Further, the residual B-cell content of the purified T-responder cells theoretically could have caused back stimulation. However this appears to be unlikely. Had the reaction observed between T-responder and T-stimulator cells been due to B-cell contamination only, the reciprocal combination should have produced a much stronger response (Table IV, exps. 2 and 3), which was not the case. For instance, B10.AQR T cells against B10.T(6R) T cells produced a stimulation index of 17.06, while the same B10.T(6R) T cells as responders against B10.AQR B cells gave only a stimulation index of 2.12 (Table IV, exp. 3). (e) In the combinations differing only in subregion I-C and in Ss,Slp (combinations 4 and 5), only T cells stimulated and no significant reaction was obtained using B-stimulator cells. This observation suggests that the I-C subregion in haplotypes t2 and t3 (Table I) may differ exclusively for T-cell surface structures. The fact that B cells do not stimulate in these combinations makes it unlikely that the stimulation obtained by T-cell fractions was due to B-cell contamination. The same may be true for combina-
tions 3 and 4 in Table IV, since in all of these cases T cells stimulated better than B cells.

Discussion

These experiments demonstrate that the I region does code for both T- and B-cell surface structures as recognized in the MLR. Whether these antigens are expressed on all cells within the T- and B-cell populations, respectively, has not been determined. Our results are to a certain extent at variance with part of the serological evidence in the literature demonstrating preponderant B-cell expression of I-region antigens (15, 16). However in those experiments the I-region gene products were tested by only Ia sera in complement-mediated cytotoxicity reactions, and the presence of nonlytic T-cell specific antibodies cannot be ruled out. Furthermore, the sensitivity of the cytotoxic reaction is greatly influenced by the method used, e.g., Frelinger et. al. using a highly sensitive microtechnique could detect anti-Ia activity directed against T cells.

The fact that, in combinations differing only in I-C and Ss,Slp, only T cells stimulated in the MLR suggests that some of the I-region genes may be preferentially expressed on T lymphocytes. A similar interpretation could apply to the experiments of Fathman and colleagues (23). They found that in the B10.A(4R) vs. B10.A(2R) combination, only B cells stimulated the MLR.

| TABLE IV |
| MLR against Purified Lymph Node T and B Cells; Responder Cells Purified |
| Lymph Node T Cells |

| Combination | Strains | Cells | Syngeneic | Allogeneic | Stimulation index | t test P< |
|-------------|---------|-------|-----------|------------|-----------------|-----------|
|             | Responder | Stimulator | cpmp ± SD | cpmp ± SD |
| 1           | C3H/DSn   | C3H.SW  | T*        | 4,536 466 | 86,383 9,502 | 19.04     | 0.001     |
|             | C3H/DSn   | C3H.SW  | T        | 13,177 4,685 | 160,135 15,413 | 12.15     | 0.001     |
|             | C3H.SW    | C3H     | T        | 2,187 295 | 63,610 3,655 | 29.18     | 0.001     |
|             | C3H.SW    | C3H     | B        | 3,260 2,163 | 209,222 18,727 | 64.18     | 0.001     |
| 2           | A.TL      | A.TH    | T        | 1,892 663 | 5,827 699 | 3.08      | 0.001     |
|             | A.TL      | A.TH    | T        | 4,009 1,446 | 4,062 1,982 | 1.91      | 0.01      |
|             | A.TH      | A.TL    | T        | 28,861 5,972 | 351,259 50,190 | 12.17     | 0.001     |
|             | A.TH      | A.TL    | B        | 27,930 3,959 | 245,968 13,301 | 8.89      | 0.001     |
| 3           | B10.T(6R) | B10.AQR | T        | 6,670 1,345 | 26,271 4,418 | 3.94      | 0.001     |
|             | B10.T(6R) | B10.AQR | T        | 4,138 1,184 | 8,783 2,859 | 2.12      | 0.05      |
|             | B10.AQR   | B10.T(6R) | T       | 2,038 655 | 35,100 7,863 | 17.06     | 0.001     |
|             | B10.AQR   | B10.T(6R) | T       | 1,080 295 | 3,919 1,441 | 3.65      | 0.02      |
| 4           | B10.HTT × | A.HT    | T        | 279 10   | 490 78 | 1.76      | 0.001     |
|             | A1F       | A.HT    | T        | 610 128  | 644 174 | 1.06      | NS§       |
|             | B10.HTT   | B10.S(7R) | T       | 3,216 590 | 5,386 673 | 1.67      | 0.05      |
|             | B10.HTT   | B10.S(7R) | T       | 4,132 1,287 | 4,460 1,290 | 1.08      | NS        |

* T, nylon wool purified lymph node T cells.
† B, anti-γ + C purified lymph node B cells.
§ NS, not significant.
Therefore, we think it is reasonable to predict that certain I-region genes will be found to be expressed preferentially on T cells and/or others on B cells. It seems possible, furthermore, that the selective expression of I-region genes may apply for T- and B-cell subpopulations as well.

Summary

Unidirectional mixed lymphocyte reactions (MLR) were performed between mouse strains differing for various segments within the H-2 complex. Thymocytes and purified lymph node T cells and B cells were used as stimulator cells. In three of five combinations studied, differing only within the I region, both T and B cells stimulated in the MLR. This suggests that the region codes for both T- and B-cell surface structures. However, if the difference was restricted to one I subregion (I-C), only T cells stimulated. This finding suggests that some of the I-region genes may be expressed either in T or in B cells.

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