

GENES FOR THE MOUSE T CELL ALLOANTIGENS Tpre,
Tthy, Tind, AND Tsu ARE CLOSELY LINKED NEAR *Igh* ON
CHROMOSOME 12

BY F. L. OWEN AND R. RIBLET

*From the Department of Pathology and Cancer Research Center, Tufts University School of
Medicine, Boston, Massachusetts 02111; and the Institute for Cancer Research,
Philadelphia, Pennsylvania 19111*

The mouse T lymphocyte alloantigens Tpre (1), Tthy (2), Tind (3), and Tsu (4) have been characterized by the development of specific monoclonal antibodies. These four alloantigens are encoded by genes on chromosome 12 near the immunoglobulin heavy chain (*Igh*) genes and are expressed on T lymphocytes at discrete stages of development (4, 1). The developmental pathway suggested by the sequential expression of these antigens does not directly parallel that marked by the Lyt-1, Lyt-2, and Lyt-3 alloantigens (5, 6). Their genetic location near the *Igh* complex and other data have raised the possibility that these four alloantigens may form part of the T cell receptor for antigen (1-8).

The expression of these four alloantigens in 26 *Igh* recombinant (IghR) and recombinant inbred (RI) mouse strains and 15 inbred strains is detailed in this paper. This study was undertaken to identify recombination between the genes coding for these antigens, to order these genes and more precisely map them on chromosome 12. In doing so, these experiments also address the question of whether these four alloantigens are products of distinct genes or instead are alternative products of a single gene. Indeed, we have found several monoclonal antibodies that bind two or more of the four antigens in various combinations (F. Owen, unpublished observations). This could indicate a structural relatedness deriving by gene duplication from a common evolutionary origin, or it could reflect a common structure modified in different cell types to create the four alloantigens. These alternatives can be distinguished by genetic test; recombination resulting in expression of only a partial set of the antigens most probably indicates individual genes for the separated antigens.

The results indicate that the expression of the four antigens is controlled by two to four genes in a tightly linked cluster distal to *Igh-I* and proximal to *Pre-1*, the locus for serum prealbumin. The map position is consistent with data obtained for *Tsu* (7) using alloantiserum and in vivo functional assays (8).

Methods

Strains of Mice. NX8 recombinant inbred (RI) strains and various *Igh* recombinant (IghR) strains (8, 9; Riblet, unpublished results) listed in Table II were bred and

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TABLE I
Expression of Tpre, Tthy, Tind, and Tsu in Inbred Strains of Mice

| Strain | Igh-1* | Tsu [‡] | Tind | Tthy | Tpre | Pre-1 [‡] |
|-------------------|--------|------------------|------------|------------|------------|--------------------|
| C.AL-20 | d | d (15 ± 3) | d (20 ± 3) | d (25 ± 6) | d (20 ± 2) | a |
| AKR/J | d | d (23 ± 2) | d (28 ± 6) | d (20 ± 3) | d (17 ± 1) | a |
| NZB | e | d (21 ± 3) | d (18 ± 2) | d (36 ± 6) | d (31 ± 5) | a |
| A/J | e | d (15 ± 1) | d (30 ± 2) | d (20 ± 2) | d (13 ± 1) | c |
| BALB/c | a | —(<1) | —(<1) | —(<1) | —(<1) | a |
| C57L/J | a | —(<1) | —(<1) | —(<1) | —(<1) | o |
| C3H/HeJ | j | —(<1) | d (15 ± 5) | d (35 ± 5) | —(<1) | c |
| NIH Swiss (Tufts) | j | —(<1) | d (22 ± 4) | d (30 ± 2) | —(<1) | o |
| CBA/J | j | —(<1) | —(<1) | —(<1) | —(<1) | a |
| C57BL/6J | b | —(<1) | —(<1) | —(<1) | —(<1) | c |
| C.B-20 | b | —(<1) | —(<1) | —(<1) | —(<1) | c |
| SWR/J | c | —(<2) | —(<2) | —(<1) | —(<1) | b |
| HRS/J | c | —(<1) | —(<1) | —(<1) | —(<1) | o |
| MRL/lpr | c | —(<1) | —(<1) | —(<1) | —(<1) | o |
| MRL/++ | c | —(<1) | —(<1) | —(<1) | —(<1) | o |

* The locus coding IgG2a immunoglobulin constant regions; typing is from Lieberman (10) and Herzenberg et al. (11).

[‡] Data are expressed as d or — (cytotoxic index ± difference from the mean). Four animals from each strain were evaluated in groups of two.

[‡] The locus controlling the electrophoretic polymorphism of serum prealbumin; typing from Taylor et al. (12) and Wilcox and Shreffler (13). The o allele denotes either b or c, not distinguished by the methodology used.

maintained at the Institute for Cancer Research, Philadelphia. The CXAXCB strains were derived from the cross, (BALB/c × A/He)F₁ × C.B-17, selecting for genetic recombination between *Igh-1* and *Pre-1*. These strains were shipped to Tufts University for short-term housing before being sacrificed. Allotype congenic and inbred strains were obtained from colonies at Tufts University or purchased from The Jackson Laboratory, Bar Harbor, ME.

Monoclonal Antibodies. Monoclonal anti-Tpre^d (F.6.9.1), Tthy^d (17IIC6), Tind^d (9IIIA2), and Tsu^d (13IIIB4) antibodies have been described (1–8). These antibodies were conjugated with fluorescein isothiocyanate (FITC) and used with mouse anti-FITC, affinity-purified polyclonal antibody, and complement to lyse cells. Visual inspection of trypan blue dye exclusion was used to evaluate the percentage of cells lysed. The methods of hapten modification, selection of positive targets, and use of cytotoxic index to evaluate data are detailed in an earlier paper (4).

Cytotoxic Index. Data are expressed as a cytotoxic index, calculated as: [(percentage of dead cells minus the percentage killed with complement)/(percentage of cells killed with monoclonal anti-Thy-1.2 minus the percentage killed with complement)] × 100. This manipulation served to normalize experiments performed on separate days. Target cells for anti-Tpre and anti-Tthy were adult thymocytes; target cells for anti-Tind and anti-Tsu were trinitrophenyl-keyhole limpet hemocyanin (TNP-KLH)-primed popliteal lymph node cells. At least four animals from each strain were evaluated and the strains were evaluated in groups over a 6-mo period.

Results

The expression of Tpre^d, Tthy^d, Tind^d, and Tsu^d on lymphoid cells from inbred strains of mice (Table I) shows general but not exclusive association of these four antigens with *Igh*. The strains of mice that express the immunoglobulin allotypes Igh-1^d or ^e (C.AL-20, AKR/J, NZB, A/J) express all four T cell alloantigens. In contrast, mice that bear the Igh-1^{a, b, or c} allotypes do not express any determinants detected by our monoclonal alloantibodies and presumably have alternative (allelic) forms of these T cell antigens. Two strains of mice, C3H/HeJ and NIH Swiss/Tufts, express Tind^d and Tthy^d but not Tsu^d or Tpre^d. These results suggest that the gene(s) controlling expression of the T cell

TABLE II
Mapping T Cell Alloantigens in RI and *IghR* Strains

| Mouse strain | <i>Igh</i> -1 | Tsu ^d | Tind ^d | Tthy ^d | Tpre ^d | Pre-1 |
|--------------|---------------|------------------|-------------------|-------------------|-------------------|-------|
| NZB | e | +(21 ± 3) | +(18 ± 2) | +(36 ± 6) | +(31 ± 5) | a |
| C57/J | a | -(<1) | -(<1) | -(<1) | -(<1) | o |
| NX8-4 | a | -(<1) | -(<1) | -(<1) | -(<1) | o |
| NX8-13A | a x | +(13 ± 1) | +(9 ± 2) | +(24 ± 4) | +(31 ± 5) x | o |
| NX8-13B | a x | +(23 ± 9) | +(14 ± 2) | +(20 ± 1) | +(18 ± 2) x | o |
| NX8-15 | e | +(16 ± 1) | +(24 ± 6) | +(28 ± 4) | +(30 ± 5) x | o |
| NX8-16 | a | -(<1) | -(<1) | -(<1) | -(<1) x | a |
| NX8-18 | e | +(28 ± 8) | +(16 ± 2) | +(10 ± 2) | +(10 ± 1) x | o |
| NX8-19 | e | +(30 ± 6) | +(18 ± 4) | +(30 ± 10) | +(15 ± 3) | a |
| NX8-20 | a | -(<1) | -(<1) | -(<1) | -(<1) x | a |
| A/lcr | e | +(9 ± 2) | +(21 ± 3) | +(30 ± 2) | +(15 ± 1) | o |
| BALB/cIcr | a | -(<1) | -(<1) | -(<1) | -(<1) | a |
| AXC-6 | e | +(18 ± 1) | +(14 ± 2) | +(13 ± 2) | +(15 ± 1) | o |
| AXC-7 | a | -(<1) | -(<1) | -(<1) | -(<1) | a |
| AXC-8 | a | -(<1) | -(<1) | -(<1) | -(<1) x | o |
| AXC-9 | a | -(<1) | -(<1) | -(<1) | -(<1) | a |
| AXC-10 | a | -(<1) | -(<1) | -(<1) | -(<1) x | o |
| AXC-11 | a x | +(25 ± 4) | +(21 ± 2) | +(10 ± 2) | +(10 ± 1) | o |
| AXC-12 | a x | +(9 ± 1) | +(16 ± 2) | +(20 ± 2) | +(33 ± 1) | o |
| BALB/cIcr | a | -(<1) | -(<1) | -(<1) | -(<1) | a |
| A/lcr | e | + | + | + | + | o |
| CXAXCB-1 | e | +(16 ± 1) | +(18 ± 1) | +(26 ± 2) | +(22 ± 2) x | a |
| CXAXCB-2 | a | -(<1) | -(<1) | -(<1) | -(<1) x | o |
| CXAXCB-3 | e x | -(<1) | -(<1) | -(<1) | -(<1) | a |
| CXAXCB-4 | e x | -(<1) | -(<1) | -(<1) | -(<1) | a |
| CXAXCB-5 | e x | -(<1) | -(<1) | -(<1) | -(<1) | a |
| CXAXCB-6 | a x | +(12 ± 2) | +(15 ± 3) | +(21 ± 3) | +(24 ± 3) | o |
| CXAXCB-7 | a | -(<1) | -(<1) | -(<1) x | +(32 ± 4) | o |
| BAB/14 | b | -(<1) | -(<1) | -(<1) | -(<1) | o |
| C.AL-9 | d | +(15 ± 3) | +(20 ± 3) | +(25 ± 6) | +(20 ± 2) | a |
| C.B.AL-1 | b | -(<1) | -(<1) | -(<1) | -(<1) x | a |
| C.B.AL-3 | b | -(<1) | -(<1) | -(<1) | -(<1) | o |
| C.B.AL-6 | b x | +(10 ± 2) | +(21 ± 6) | +(16 ± 1) | +(10 ± 1) | a |

Alloantigen expression is indicated as + or -; in parentheses is the cytotoxic index \pm difference from the mean. Crossover points are indicated by an x.

alloantigens are linked to the *Igh* locus, and that a recombination event occurred between the gene(s) controlling Tind and Tthy expression and the gene(s) controlling Tsu and Tpre before development of the C3H and NIH Swiss mouse strains.

A panel of 26 RI and *IghR* strains were studied for the expression of the Tpre, Tthy, Tind, and Tsu alloantigens (Table II). Four separate groups of strains of different genetic backgrounds were studied. The two parental strains used to construct the recombinant strains are listed first in each panel of Table II. The position of recombination events in and around the immunoglobulin locus is indicated.

In the NX8 RI strains, genetic recombination has occurred between the T cell alloantigen genes and the prealbumin locus, *Pre-1*, in strains NX8-15, 16, 18, and 20. The 13A and 13B strains are double recombinants with a crossover on each side of the T cell antigen gene cluster.

Crossovers between the immunoglobulin gene cluster and the T cell gene cluster also occurred in two of the AXC strains of mice (AXC-11, 12). Two other



FIGURE 1. Genetic map of mouse chromosome 12. The centromere is at the left. Ly-16, originally designated Ly-18 (16), has been renumbered in accordance with the decisions of an ad hoc committee for the clarification of Ly antigen nomenclature (E. Boyse, U. Hammerling, D. Meruelo, and F. W. Shen; I. F. C. McKenzie and H. C. Morse, chairmen). The order and spacing of Ly-16 and Lyb-7 is not known.

strains show recombination events between the T gene cluster and the prealbumin locus (AXC-8, 10).

The CXAXCB-7 mouse defines the position of the gene coding for Tpre relative to the other T cell genes and to *Pre-1*. This strain shows a crossover between *Tpre* and the other three T genes. Since the *Pre-1*^o allele and the *Tpre* alleles are derived from the A/Icr parent, this crossover places *Tpre* distal to the other T genes, between them and *Pre-1*. Two additional strains separate the T cell genes from *Pre-1* (CXAXCB-1 and -2) and four strains separate the T cell markers from the *Igh* genes (CXAXCB-3, -4, -5, and -6).

The C.B.AL series should be broadly useful, as these strains can also be used for analyzing analogous T antigens (*T^b*) identified in *Igh^b* haplotype mice (14, 15). The C.B.AL-1 and C.B.AL-6 mice have recombination events that bracket the *T^d* genes; if the *T^b* genes map to this same interval, this will support the presumption that these are alleles of the *T^d* genes analyzed here. The AKXL-24 strain was tested and the results were consistent with earlier data (8) showing that this strain fails to express the *T^d* antigens, due to a crossover between *Igh-1* and the T gene cluster (data not shown).

The studies in this paper confirm earlier studies (8) that used alloantiserum and functional, in vivo assays to map *Tsu* to a short segment of chromosome 12, as shown in Fig. 1. These studies also show that all four T cell alloantigen genes are clustered in this region. We have identified nine instances of genetic recombination between *Igh*, the immunoglobulin heavy chain gene complex, and the cluster of T antigen genes; one crossover within the cluster and 13 crossovers (including AKXL data [8]) between the T cluster and *Pre-1*. As the recombination frequency is 10% in the *Igh-C--Pre-1* interval, our data indicates the following genetic organization: *Igh-C--4%--(Tthy, Tind, Tsu)--0.4%--Tpre--6%--Pre-1*.

The order of Tthy, Tind, and Tsu is not established by the analysis of *Igh* recombinant strains, but if the expression in C3H/HeJ and NIH Swiss mice of only Tind^d and Tthy^d does reflect a recombinant genotype, then Tsu is also uniquely ordered [(Tthy, Tind)--Tsu--Tpre], although no distance estimate can be made.

The genetic separation of Tpre from Tsu from Thy and Tind demonstrates the existence of at least three genes controlling the expression of these determinants. We believe that Tthy and Tind are also controlled by separate genes because they are expressed on different subpopulations of T cells. Although it is formally possible that some or all four of these antigens are structural modifications of a single gene product, we favor the interpretation that these alloantigens are a set of functionally differentiated cell surface molecules coded by a cluster of four homologous genes that arose by duplication and divergence. They may contribute to different functional forms of a complex receptor for antigen.

Summary

Genes encoding the T cell alloantigens Tpre, Tthy, Tind, and Tsu have been mapped to a short segment of chromosome 12 using *Igh* recombinant strains of mice. These loci are located in a tightly linked cluster between the immunoglobulin heavy chain constant region gene cluster, *Igh-C*, and the serum prealbumin locus *Pre-1*, in this manner: centromere---*Igh-C*---(*Tthy*, *Tind*)-*Tsu*-*Tpre*---*Pre-1*.

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