The Ly-15 alloantigenic system: A genetically determined polymorphism of the murine lymphocyte function-associated antigen-1 molecule

(cell membrane molecule/Ly antigens)

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ABSTRACT Serological and biochemical studies using monoclonal antibodies have demonstrated that the Ly-15 cell membrane alloantigens are polymorphic sites on the lymphocyte function-associated antigen-1 (LFA-1) molecule. Ly-15.2 and LFA-1 show identical tissue distributions, being present on all thymocytes, lymphocytes, and neutrophils, and show cytotoxicity. Furthermore, anti-Ly-15.2 and anti-LFA-1 antibodies coprecipitate the same molecule from lymphocyte lysates, and peptide mapping shows that the Ly-15.2 and LFA-1 α chains are identical, as are the β chains.

Antibodies have been used to define several immunologically important cell membrane structures on effector T lymphocytes in mice. These are (i) the T-cell receptor complex (i.e., antigen receptor and associated molecules including T3 and its putative equivalent in mouse) (1, 2), (ii) the Ly-2/3 molecule (3, 4), (iii) L3T4 (5), and (iv) lymphocyte function-associated antigen-1 (LFA-1) (6–8). The importance of these cell membrane molecules in T-cell function is seen from experiments in which specific antibodies binding to these molecules enhance or inhibit a particular function (1–8). The precise mechanism of enhancement or inhibition is not fully understood, but in the case of LFA-1, LFA-1 antibodies inhibit T-cell killing by preventing effector/target cell conjugation (7, 8). Thus, it is believed that the LFA-1 molecule acts by assisting in the stable attachment of the cytotoxic T cell to its target. Biochemical studies of the murine LFA-1 molecule have shown it to be a heterodimer composed of a M, 180,000 α chain non-covalently associated with a M, 95,000 β chain (9). Furthermore, other studies have demonstrated that the LFA-1 molecule is related to the Mac-1 molecule, which is present only on granulocytes and macrophages and is the C3bi receptor (10). Mac-1 is a heterodimer of M, 177,000 α chain non-covalently bound to the same M, 95,000 β chain that is part of the LFA-1 complex; therefore, LFA-1 and Mac-1 comprise a family of immunologically important molecules (8, 11).

Many genes encoding cell-membrane alloantigens have been described in the mouse and include the major histocompatibility complex, immunoglobulin genes, and the Ly loci. At present, there are 30 known Ly loci, which are defined by antibodies recognizing polymorphic forms of cell-membrane alloantigens (12, 13) in contrast to most xenoantibodies (e.g., LFA-1 antibodies) that recognize a monomorphic structure—i.e., all strains of mice are LFA-11 and no polymorphism of this molecule has been demonstrated. The anti-Ly antibodies have been useful for defining and manipulating subsets of lymphocytes (12, 13), but the reason why some antigens are selectively expressed is unclear and, indeed, the function of no Ly antigen is fully understood. A typical example of a Ly alloantigenic system is the Ly-15 locus, which was originally described as having two alleles coding for the cell-membrane antigens, Ly-15.1 and Ly-15.2, each identified by antibody. These specificities were detected on thymocytes and all T and B cells. The Ly-15 locus has not been mapped nor is its function known (14).

We now describe serological and biochemical studies of the molecules identified by anti-Ly-15.2 and LFA-1 antibodies and show that the antibodies to Ly-15 alloantigens detect a polymorphic site on the LFA-1 molecule.

MATERIALS AND METHODS

Mice. Mice used are listed in Table 1. They were bred and maintained at the Research Centre for Cancer and Transplantation (The University of Melbourne) or The Jackson Laboratory.

Antisera and Serological Assays. Monoclonal antibodies used were anti-Ly-15.2, 8-6-2 (14), anti-LFA-1, M14/7 (6), anti-Ly-2.1, 49-11.1 (15), and monomorphic anti-Ly-1 53-6-72 (16). Ly-15.2 or Ly-2.1 antibodies were from ascites fluid; anti-LFA-1 or Ly-1 antibodies were obtained from culture supernatant.

Binding antibodies to cells was assessed (i) by indirect immunofluorescence using an Ortho 50H cytofluorograph to measure fluorescence intensity (17); (ii) by rosetting, using sheep anti-mouse Ig-conjugated indicator cells (18); and (iii) by binding of radiolabeled antibodies (19). Briefly, Ly-15.2 antibody was purified using Staphylococcus aureus Protein A Sepharose (Pharmacia) and labeled with Na125I, using chloramine-T.

Binding assays were conducted in 96-well microtiter plates by mixing cells (5 × 10⁵) suspended in phosphate-buffered saline containing 0.5% bovine serum albumin with 125I-labeled Ly-15.2 antibody (12.5–50 μg) in a total reaction volume of 100 μl. Suspensions were incubated for 30 min at 4°C and washed repeatedly in phosphate-buffered saline containing bovine serum albumin. Plates were dried, and the radioactivity of individual wells was determined in a radiation counter.

Cells. Thymus, lymph node, spleen, and bone marrow cells were prepared in phosphate-buffered saline containing bovine serum albumin. Ig⁺ lymph node T cells were prepared by rosetting Ig⁺ B cells with sheep anti-mouse Ig-coat-
ed erythrocytes and depleting rosetted cells on isopaque Ficoll gradient (20). Calcium caseinate-induced neutrophils, induced by intraperitoneal injection of 0.4% calcium caseinate, were purified by density gradient centrifugation (21).

**Immunochromical Analysis.** C57BL/6 thymocytes were labeled with 125I using lactoperoxidase and were solubilized in buffer containing Triton X-100 (17). Immunoprecipitations were performed by reaction of lysates with antibodies (5 μl of ascites or 50 μl of supernatant) and by precipitating the immune complexes with rabbit anti-mouse Ig conjugated to Sepharose 4B (Pharmacia). Two-dimension gels (IEF, first dimension; 7.5% NaDodSO4/PAGE, second dimension) were run under reducing conditions as described (17). Co-precipitation studies of Ly-15.2 and LFA-1 were carried out by adding to lysate samples (5 × 10⁶ cell equivalents) a large excess of anti-immunoglobulin Sepharose 4B conjugate (Pharmacia 4B) to which an excess of specific monoclonal antibodies or normal mouse Ig had been bound. Three sequential immunodepletions were carried out for each monoclonal antibody. Peptide mapping studies on radioactive protein bands were performed by excising the appropriate band from NaDodSO4/polyacrylamide gel and placing the dried gel strips into loading chambers of a NaDodSO4/15% polyacrylamide gel. The strips were rehydrated in situ in digestion buffer containing 50 μg of S. aureus V8 protease per ml (Miles). Subsequent steps were performed as described by Cleveland et al. (22).

**RESULTS AND DISCUSSION**

**Ly-15 Locus.** This was originally described in the mouse as a polymorphic two-allele alloantigenic locus (14). Further analyses have been undertaken in this study, and additional strains of mice have been tested using the monoclonal anti-

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### Table 1. Strain distribution of the Ly-15.1 and Ly-15.2 specificities

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ly-15.2 (Ly-15.1+)</th>
<th>Ly-15.2*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>AKR, BALB/cBy, C58, LT/J, NZB/BinJ, PL/J, SJL, SWR, 129/ReJ</td>
<td>A, C57L, C57BL/KsJ, C57BR/cD, C3H/HeJ, CBA, C57BL/6 and related strains congeneric for Ly-1, Ly-2, Ly-3, Ly-5, Ly-9, Qa, Thy-1; C57BL/10 and related strains congeneric for H-3/Ly-4, H-2b, H-2k; C3H.B6-Ly-6*, L.RIII, A.Thy-1*</td>
</tr>
<tr>
<td>Congeneic</td>
<td>BALB-H-2*, BALB-H-2a</td>
<td></td>
</tr>
</tbody>
</table>

Recombinant inbred (progenitor strains)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ly-15.2 (Ly-15.1+)</th>
<th>Ly-15.2*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXB (C57BL/cBy × BALB/6By)</td>
<td>CXBE, G, K</td>
<td>CXBD, H, I, J</td>
</tr>
<tr>
<td>SWXL (SJR × C57L/J)</td>
<td>SWXL-7, 12, 14, 16, 17</td>
<td>SWXL-4, 15</td>
</tr>
<tr>
<td>NX129 (NZB/BinJ × 129/J)</td>
<td>NX129-7, 19, 12</td>
<td>NX129-1, 5</td>
</tr>
<tr>
<td>LXPL (C57L/J × PL/J)</td>
<td>LXPL-6</td>
<td>LXPL-1, 4</td>
</tr>
</tbody>
</table>

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### Table 2. Tissue distribution of Ly-15 and LFA-1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% cells expressing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ly-15.2</td>
</tr>
<tr>
<td>Thymus</td>
<td>90</td>
</tr>
<tr>
<td>Spleen</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Lymph node</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>75</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>&gt;95</td>
</tr>
</tbody>
</table>

% antigen positive cells in C57BL/6 mice were determined using SAMIg-rosetting. Surface Ig was removed from B cells in spleen, lymph node, and bone marrow cell suspensions by capping (18).

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**Fig. 1.** Flow cytometric analysis of C57BL/6 thymocytes and Ig- lymph node T cells treated with anti-Ly-15.2 (A and B, respectively) or anti-LFA-1 (C and D, respectively). Expression was quantitated using indirect immunofluorescence and an orthocytometer. Background staining with an isotype-matched negative antibody (anti-Ly-2.1 for Ly-15.2, rat anti-epithelial growth factor for LFA-1) is shown as (——). Scaling on both axes is linear.
Ly-15.2 antibody (Table 1). Typing of 22 recombinant inbred (RI) strains, which have been used to map many murine genes (23), was not informative in determining the chromosomal location of the Ly-15 gene, as there was no co-segregation of Ly-15 alleles and other known markers in these strains. In addition, the Bailey bilineal congenic strains were tested for possible linkage of Ly-15 to 27 histocompatibility genes (derived from BALB/c (Ly-15.2+)) and placed on C57BL/6 (Ly-15.2−) genetic background (23), but all strains showed the C57BL/6 phenotype. Thus, linkage to H-1, -2, -3, -7, -8, -15, -16, -17, -18, -19, -20, -21, -22, -23, -24, -25, -26, -27, -28, -29, -30, -36, -37, -38, and 4.2 other unnamed H loci was excluded (data not shown). However, despite the testing of more inbred, congenic, and recombinant inbred stocks, the location of the Ly-15 gene is still unknown.

Tissue Distribution. The tissue distribution of Ly-15.2 and LFA-1, examined by rosetting and flow cytometry, showed a striking similarity. Both Ly-15.2 and LFA-1 were found on >95% of thymus, lymph node, and spleen cells and, therefore, all T (Thy-1+) and B (Ig+ cells) cells in these tissues (Table 2); they were also present on most bone marrow cells and calcium caseinate-induced neutrophils. Furthermore, flow cytofluorometric analysis demonstrated that Ly-15.2 and LFA-1 expression was identical on thymocytes and also lymph node T cells (Fig. 1). More than 90% of thymocytes expressed only low levels of Ly-15.2 and LFA-1, but the fluorescence profiles were identical with a discrete peak at channel 1.4 for both antibodies (Fig. 1 A and C). Similarly, most lymph node T cells expressed low levels of Ly-15.2 and LFA-1, and these profiles were also identical. It should also be noted that an additional population of cells expressed relatively higher amounts of both of these antigens (more than channel 2.2) (Fig. 1 B and D). These cells constituted 16% of all Ly-15.2 cells and 12% of LFA-1+ cells. Thus, both the tissue distribution and expression of Ly-15.2 and LFA-1 on thymus and lymph node cells are identical and both antigens are similarly distributed in other tissues (Table 2).

Immunofluorescence Analysis. One-dimensional NaDodSO4/PAGE indicated that Ly-15.2 and LFA-1 had a similar two-chain molecular structure (data not shown) and the possible identity of the Ly-15.2 and LFA-1 molecules was directly addressed by two-dimensional NaDodSO4/PAGE, peptide mapping, and coprecipitation.

(i) Two-dimensional gels. The LFA-1 and Ly-15.2 molecules show the same subunit structure, each being composed of a α, 180,000 α chain and a β, 94,000 β chain (Fig. 2). The Ly-15.2 α or β chains also had an identical pl when compared to the LFA-1 α and β chains. Indeed, in an experiment in which Ly-15.2 and LFA-1 immunoprecipitates were mixed prior to electrophoresis, the patterns of the α and β chains were indistinguishable from Ly-15.2 or LFA-1 alone—i.e., both antisera precipitate molecules of the same size and charge.

(ii) Peptide mapping studies. These studies confirmed that the Ly-15.2 and LFA-1 molecules are related. LFA-1 and Ly-15.2 α chains yielded identical partial cleavage peptides after limited proteolysis with S. aureus V8 protease (Fig. 3). Similarly, no differences between the β chains were seen in the peptide maps. It should be noted that the α and β chains were clearly different from each other, and from the Pgp-1 molecule (a major leukocyte glycoprotein (24)) included for control purposes.

(iii) Coprecipitation studies. The data from the two-dimensional gels and peptide maps were consistent with the possibility that the Ly-15.2 and LFA-1 molecules were closely related or identical structures. Thus, coprecipitation studies
Fig. 3. Cleveland peptide mapping of Ly-15.2 and LFA-1 α (heavy) (lanes A and B) and β (light) chains (lanes C and D) and the Pgp-1 molecule (lane E). Peptides were resolved on a 15% NaDodSO₄/polyacrylamide gel. Numbers on left represent \( M_r \times 10^{-3} \).

were performed to demonstrate that the Ly-15.2 and LFA-1 antigenic determinants reside on the same molecular complex. Lysates from C57BL/6 thymocytes labeled with \(^{32}P\)I were precleared with normal mouse Ig (Fig. 4A), anti-Ly-15.2 or anti-LFA-1 antibodies (Fig. 4B and C, respectively). The appropriate α and β chains were precipitated by anti-Ly-15.2 and anti-LFA-1 antibodies after preclearing with normal mouse Ig (Fig. 2). It should also be noted that a trace of material of \( M_r, 170,000 \) is also seen, and preliminary evidence indicates that this is the precursor of the \( M_r, 180,000 \) LFA-1 α chain. In addition, a trace of an α chain breakdown product of \( M_r, 116,000 \) may be observed. Preclearance of the Ly-15.2 antigen resulted in the removal of most but not all of both Ly-15.2 and LFA-1 molecules (Fig. 4B). In the reciprocal experiment, removal of the LFA-1 antigen also resulted in the removal of Ly-15.2 molecules (Fig. 4C). The failure to remove all Ly-15.2 and LFA-1 molecules by preclearance with anti-Ly-15.2 and LFA-1 molecules by preclearance with anti-Ly-15.2 in contrast to total preclearing with anti-LFA-1 is most probably a reflection of qualitative differences in preclearing procedures with different antibodies. Notwithstanding, precipitation with anti-Ly-15.2 removes the vast majority of Ly-15 and LFA-1 molecules. These reciprocal immunodepletions were specific, as there was no depletion of the Ly-1 molecules in any precleared lysate (Fig. 4, lanes b).

Thus, the epitopes detected by the anti-Ly-15.2 and LFA-1 antibodies are present on the same structure. However, the relationship between the Ly-15.2 and LFA-1 epitopes is not clear, but as anti-Ly-15.2 antibodies detect a polymorphic site, whereas the anti-LFA-1 antibody detects a monomorphic site (common to all strains of mice), it is likely that they recognize different epitopes. Indeed, preliminary experiments indicate that anti-LFA-1 antibodies do not block the binding of anti-Ly-15.2 antibodies and anti-Ly-15.2 antibodies do not inhibit T-cell killing (unpublished), demonstrating that the two sites are probably distinct.

As LFA-1 is a heterodimeric molecule, the Ly-15 alloantigen could represent polymorphism of either the α chain or the β chain, or they may arise as a combinatorial determinant by association of different allelic forms (not serologically defined) of the α and β chains. Indeed, polymorphisms of individual proteins in multimeric molecules are seen in H-2 (β-2 microglobulin and H-2) (25, 26), immunoglobulin (heavy and light chains) (27, 28), and Ly-2/3 (29). Combinatorial polymorphisms have also been described for H-2 (30), Qa-2 (31), and Ia (32), but at present it is not clear whether the Ly-15.2 epitope is chain specific or combinatorial. Most anti-LFA-1 monoclonal antibodies detect epitopes present on the α chain and it will be important to determine the chain location of the Ly-15.2 epitope, as one major advantage of alloantibodies is their use in gene mapping studies. LFA-1 is one member of a family of related heterodimersic molecules that all share the same β chain. These include Mac-1 (C3bi receptor) in the mouse, and in humans, LFA-1 (33, 34), OKM1 (C3bi receptor that is also detected by the murine Mac-1 antibody) (35), and gp 150,95, the function of which is unknown (34, 35). At present, the location of the genes coding for either α or β chains is unknown, but the use of antibodies directed against polymorphic determinants of these chains will assist gene mapping studies yielding information required to understand the genetic and structural relationships between LFA-1 and related molecules.


