DISSECTION OF THE B10.D2 ANTI-H-2Kb CYTOLYTIC T LYMPHOCYTE RECEPTOR REPERTOIRE*

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The immune potential of an individual is determined by the receptor repertoire available to numerous interrelated lymphocyte subpopulations. Until recently, most research dealing with repertoire has been conducted in the B lymphocyte compartment. These studies have taken advantage of the fact that the immunoglobulin product of a B cell is idiotypically identical to the cell's receptor (1). Methods that have been used to distinguish immunoglobulins, and thereby define repertoire, including (a) amino acid sequence analysis, (b) fine specificity analysis or affinity of antibody, (c) reactivity with anti-idiotypic reagents, and (d) isoelectric focusing of antibodies (2).

In contrast, because of the inaccessibility of the T cell receptor, relatively little is known about the T cell repertoire. Conclusions regarding T lymphocyte receptor diversity have been largely based on the study of the specificity of antigenically stimulated populations of T cells. This approach has been applied to the study of most T cell subpopulations and is the basis of the belief that the receptors found on T cells exhibit a high degree of antigen specificity (3-5). This approach has also revealed several features that appear to be unique to T lymphocyte function. For example, in addition to antigen specificity, T cells are functionally restricted to H-2-identical cells (6-10). Also, a high proportion of T cell precursors are activated by H-2 alloantigens (11-16). It is not known to what extent these properties are reflected in the T cell receptor repertoire; however, they introduce the possibility of a profound effect of H-2 on repertoire selection, a feature not usually associated with the B cell repertoire (17-22).

On the other hand, studies with anti-idiotypic reagents prepared against immunoglobulin idiotypes have revealed similarities between T and B cell receptors. B cell idiotypes have been demonstrated on a variety of different T cell subsets (23-29). Receptors on murine cytolytic T lymphocytes (CTL),1 however, have not as yet been found to bear B cell-idiotypic determinants (30).

Resolution of questions concerning the extent of sharing between the T and B cell compartment, as well as genetic or environmental determination of the T cell repertoire, require an experimental approach that could identify the entire T cell repertoire specific for a particular antigen. One approach that was proven useful in identification of the B cell repertoire is the application of panel analysis to the

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1 Abbreviations used in this paper: Con A, concanavalin A; CTL, cytolytic T lymphocyte(s); Kh, C57BL/6Kh; TCGF, T cell growth factor.
immunoglobulin product of monoclonal B cells (31, 32). Recent technological advances permit application of an analogous approach to study the CTL receptor repertoire. There are currently two methods available that produce monoclonal CTL. One is antigenic stimulation of CTL precursors that are clonally distributed by limiting dilution (15, 16). This method permits determination of the frequency of antigen-specific precursors. It has the disadvantage of providing a limited number of clonal progeny that is insufficient to permit clonal identification by panel analysis. The second method used to obtain CTL clones entails multiple stimulation of a population that contains numerous precursors, followed by cloning in the presence of T cell growth factor (TCGF) obtained from stimulated T cells (33, 34). This method has the advantage of providing CTL clones that may be continuously expanded in the presence of TCGF, and, therefore, such clones would presumably permit panel analysis of specificity. However, this method has the disadvantage of not providing information concerning the representation of each clonotype in the precursor repertoire.

In this report, an experimental approach is described that combines these two methods in a manner compatible with repertoire analysis. Antigenically stimulated CTL clones are obtained by limiting dilution and subsequently expanded with the aid of TCGF. Such clones are then subjected to panel analysis. This strategy has been applied to the CTL receptor repertoire specific for an H-2 alloantigen, H-2K\textsuperscript{b}. This particular antigen was chosen for several reasons: (a) the high frequency of monoclonal CTL specific for alloantigens (15, 16), (b) there are numerous H-2K\textsuperscript{b} mutants that express many different determinants that can be used as the panel necessary to identify individual clones (35), and (c) previous studies employing monolayer absorption have demonstrated the feasibility of using these mutants to discriminate CTL subpopulations (36).

This report describes the specificity repertoire of the anti-H-2K\textsuperscript{b} response in B10.D2 animals. Dissection of the repertoire by panel analysis has revealed the existence of a minimum of ~50 unique receptor specificities directed against different antigenic determinants present on the H-2K\textsuperscript{b} molecule. Each specificity is represented at a frequency of <1/18,000 CTL precursor cells. The implications of a diverse repertoire are discussed from the point of view of the high frequency of alloreactive T cells.

With respect to the H-2K\textsuperscript{b} molecule, these studies have identified a minimum of 23 antigenic determinants and describe their distribution amongst the 7 mutants considered in these analyses.

Materials and Methods

Animals. H-2 mutants are designated with recommended nomenclature (37). C57BL/6Kh, B6.H-2\textsuperscript{bm3}, B6.C-H-2\textsuperscript{bmst}, B6.H-2\textsuperscript{bm8}, B6.C-H-2\textsuperscript{bm9}, B6.C-H-2\textsuperscript{bm10}, and B6.C-H-2\textsuperscript{bm11} were kindly provided by Dr. Roger Melvold and Dr. Henry Kohn (Harvard Medical School, Boston, Mass.) and subsequently bred in the Scripps Clinic and Research Foundation breeding colony (La Jolla, Calif.). B6.C-H-2\textsuperscript{bm4} were also obtained from Dr. Donald Bailey (The Jackson Laboratory, Bar Harbor, Maine). All other strains of mice were obtained from the Scripps Clinic and Research Foundation breeding colony. Animals used as a source of responder lymphocytes were 8-12 wk old. 6-mo-old male Wistar rats used in the preparation of TCGF were purchased from Microbiological Associates, Walkersville, Md.

Preparation of Cells. All spleen cell suspensions were prepared by teasing spleens in minimal essential media that contained 2% fetal bovine serum (Grand Island Biological Co., Grand Island, N. Y., or Sterile Systems, Inc., Logan, Utah), 5 μg/ml gentamycin, and 1 mM glutamine.
Debris was removed by passage through a Nytex filter (The Firestone Tire & Rubber Co., Akron, Ohio). Spleen cells used as stimulator and helper cell populations were irradiated for 25 min at 94 rad/min with a $^{137}$Cs source. Either before or after irradiation erythrocytes were lysed by incubation for 2 min at 37°C in 0.17 M ammonium chloride that contained 10 mM Tris-HCl (pH 7.2).

Primary In Vitro Stimulation of Monoclonal CTL. The method used to obtain monoclonal CTL is adapted from that of Lindahl and Wilson (15) and Teh et al. (16). $3 \times 10^5$ irradiated B10.D2 helper cells plus $1 \times 10^6$ B6 or B10.A3R or B10.A5R stimulator cells resuspended in RPMI-1640 that contained 25% fetal bovine serum, 1 mM glutamine, 5 μg/ml gentamycin, and 5 $\times$ $10^{-6}$ M 2-mercaptoethanol (culture media) were added to wells of 96-well V-bottom Linbro plates (Linbro Chemical Co., Hamden, Conn.). The indicated number of responder B10.D2 spleen cells were added in 0.1 ml in this same media. Plates were incubated at 37°C in an atmosphere of 5% CO$_2$ in air.

Detection of CTL Clones. After 6 d of incubation, culture plates were replicated by transferring 100 μl of cell suspension to fresh plates with a 12-channel multwell pipetter (Flow Laboratories, Inc., Rockville, Md.). CTL clones were detected with $^{125}$I-labeled EL-4 target cells (H-2$^b$) that were labeled for 2 h at $1 \times 10^6$ cells/ml in culture media that contained 10% fetal bovine serum, $1 \times 10^{-6}$ M fluorodeoxyuridine (Sigma Chemical Co., St. Louis, Mo.), and 1 μCi/ml $[^{125}I]$iododeoxyuridine (Amersham Corp., Arlington Heights, Ill.). Cells were then washed three times and resuspended at $1 \times 10^7$/ml in culture media that contained 10% fetal bovine serum and incubated for 18–22 h at 37°C. After this time, 10 μl of media that contained 2.5 μg of deoxyribonuclease I (Sigma Chemical Co.) was added to each well, and the plates were further incubated for 1 h at 37°C. The plates were then spun at 1,200 rpm for 10 min, 100-μl aliquots were removed from each well, and radioactivity was determined with a gamma counter (model 28004, Micromedic Systems, Horsham, Pa.). Wells were considered positive for the presence of a CTL clone if isotope release was 2 SD above control values obtained from wells that did not receive responder cells. Spontaneous release of EL-4 target cells was 8–20%.

Expansion of Monoclonal CTL. Cells and media from positive wells were added to 0.7 ml of culture media supplemented with 10% partially purified rat TCGF in conical centrifuge tubes (2095, Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). TCGF was prepared by the methods of Gillis et al. (38) and partially purified as follows: Culture supernate was brought to 0.1 M in α-methyl-D-mannoside (Sigma Chemical Co.) and then 40% of saturation with solid ammonium sulfate. After removal of the resulting precipitate, the supernate was brought to 70% of saturation with ammonium sulfate. The precipitate was collected and dialyzed overnight against minimal essential media that contained 10 mM of Hepes buffer (pH 7.4). Precipitated material was removed by centrifugation, and the supernate was applied to a column of Sephadex G-100 (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.). The activity of each fraction was assayed for its ability to support the development of a primary BALB/c anti-C57BL/6 monoclonal response in limiting-dilution culture in the absence of helper cells. Under these conditions, when $4 \times 10^3$ lymphocytes are added per well, responsiveness is totally dependent upon the presence of TCGF.

Determination of Clonal Expansion. After 5 d in culture, a 50-μl aliquot of each clone was added to $1 \times 10^4$ $^{51}$Cr-labeled EL-4 tumor cells. Cells were labeled by incubation of $2 \times 10^6$ cells for 2 h in 0.1 ml of culture media plus 0.1 ml of $^{51}$Cr-sodium chromate (New England Nuclear, Boston, Mass.). Percent specific lysis was determined by the formula:

$$\frac{\text{sample (counts per minute) - spontaneous release (counts per minute)}}{\text{maximum release (counts per minute) - spontaneous release (counts per minute)}} \times 100.$$ 

Spontaneous release for target cells ranged from 8 to 15%.

Panel Analysis of Expanded Clones. After 6 d in culture, each clone received 1.5 ml of culture media and was distributed in 0.1-ml aliquots into wells of microtiter plates to assess lytic potential on each of nine different target cells. Assays on each target were performed in duplicate. Target cells were concanavalin A (Con A) (Miles Laboratories, Inc., Elkhart, Ind.) blasts labeled with either $^{51}$Cr or $^{125}$I as described above. Spontaneous release in all experiments was 15–25% for $^{125}$I-labeled cells after overnight incubation, and 20–30% for $^{51}$Cr-labeled cells after 4 h of incubation.
Blast Cells for Panel Analysis. Target cells were prepared by incubation of spleen cells for 2 d in culture media that contained 10% fetal bovine serum and 2.5 μg/ml Con A.

Results

Frequency of B10.D2 Anti-H-2K\textsuperscript{b} Clones. The analysis of receptor specificity assumes that each clone represents the progeny of a single precursor cell. Therefore, it was necessary to determine the frequency of precursors developed against the H-2K\textsuperscript{b} molecule in primary limiting-dilution cultures. Varying dilutions of B10.D2 responder spleen cells were stimulated with either B10.A3R or B10.A5R (H-2K\textsuperscript{b} and H-2D\textsuperscript{b}) cells as described in Materials and Methods. The results obtained for the average of seven different experiments are shown in Fig. 1. The frequency of clones is proportional to the number of responder cells added per well as predicted for a random distribution, which indicates that under the culture conditions employed, the CTL precursor is the only limiting cell. The averaged frequency of anti-H-2K\textsuperscript{b} precursors is 1 in \(12 \times 10^3\) spleen cells. This frequency is comparable to previously obtained estimates of the frequency of CTL precursors specific for H-2K (39). All clones used in the experiments described below were developed with 2,500 responder cells/well to assure a high probability of monoclonality.

Expansion of Monoclonal CTL. Several conditions were tested for expansion of monoclonal CTL. Cells and culture media from wells previously assessed as positive for the presence of a CTL clone were added to 0.7 ml of culture media that contained either 10% TCGF, cells bearing H-2K\textsuperscript{b} antigens, or both. After 5 d of culture, aliquots from each clone were tested for the ability to lyse \(^{51}\text{Cr}\)-labeled EL-4 tumor cells. The results shown in Table I indicate that TCGF (but not stimulator cells) are required for adequate propagation of clones. In most experiments, between 10 and 50% of clones successfully expanded with these conditions. Clones were considered sufficiently expanded to use in panel analysis if they could lyse 15% or more of target cells.

![Fig. 1: Frequency of anti-H-2K\textsuperscript{b} CTL precursors in the B10.D2 spleen. 24 cultures, each with the indicated number of B10.D2 spleen cells, were stimulated with B10.A3R- or B10.A5R-irradiated spleen cells as described in Materials and Methods. Cultures were assayed on \(^{51}\text{Cr}\)-labeled EL-4 target cells. The results represent the average for seven individual B10.D2 donors in seven different experiments. The line was obtained by the least squares method. The correlation coefficient, \(r = 0.99\). The extrapolated value, which yields 37% negative cultures, indicates the frequency of anti-H-2K\textsuperscript{b} CTL precursors. This value is 1 in \(11.7 \times 10^3\) spleen cells.](image-url)
**Table I**

| Addition to culture media                  | Fraction of clones successfully expanded |
|--------------------------------------------|------------------------------------------|
| 4 x 10⁶ cells*                             | 0/15                                     |
| 10% TCGF                                   | 20/34                                    |
| 10% TCGF + 4 x 10⁶ cells                   | 7/13                                     |

* Cells were (B6 X DBA/2)Frirradiated spleen cells. 50-μl aliquots removed from each clone were incubated with ³¹Cr-labeled EL-4 tumor cells. Lysis was detected in a 4-h ³¹Cr-release assay. Clones were considered expanded if lysis exceeded 15%.

**Table II**

Examples of Panel Analysis of Clones from Two Individual B10.D2 Donors

| Donor | Clone | Percent specific isotope release* | Assigned reactivity pattern |
|-------|-------|----------------------------------|-----------------------------|
|       |       | Target Cell                      | D2.GD                       |
|       |       | Kh     | bml8 | bml1 | bml3 | bml4 | bml9 | bml10 | bml11 | Kh     | bml8 | bml1 | bml3 | bml4 | bml9 | bml10 | bml11 |
| A     | 1     | 27    | 3    | 96   | 5    | 41   | 8    | 7    | 2     | +     | -     | -     | +     | -     | -     | -     | -     |
|       | 2     | 54    | 13   | 23   | 53   | 9    | 12   | 25   | 40    | 6     | Partial reactivity on bml1 and bml10 |
|       | 3     | 25    | 3    | 0    | 0    | 4    | 0    | 3    | 9     | 7     | +     | -     | -     | +     | +     | +     | +     | +     |
|       | 4     | 33    | 9    | 6    | 45   | 6    | 11   | 11   | 39    | 7     | +     | -     | -     | +     | -     | -     | +     | +     |
|       | 5     | 31    | 11   | 5    | 32   | 38   | 30   | 23   | 43    | 8     | +     | -     | -     | +     | +     | +     | +     | +     |
|       | 6     | 77    | 24   | 16   | 24   | 50   | 55   | 50   | 19    | 13    | +     | -     | -     | +     | +     | +     | -     | +     |
| B     | 1     | 45    | 5    | 9    | 6    | 8    | 53   | 13   | 10    | 8     | +     | -     | -     | +     | -     | +     | +     | -     |
|       | 2     | 33    | 10   | 16   | 13   | 16   | 55   | 11   | 16    | 4     | +     | -     | -     | -     | -     | -     | -     | -     |
|       | 3     | 52    | 6    | 2    | 2    | 2    | 58   | 5    | 4     | 5     | +     | -     | -     | +     | -     | -     | -     | -     |
|       | 4     | 45    | 0    | 2    | 1    | 0    | 39   | 3    | 4     | 2     | +     | -     | -     | -     | -     | -     | -     | -     |
|       | 5     | 53    | 4    | 10   | 4    | 3    | 51   | 9    | 9     | 8     | +     | -     | -     | -     | -     | -     | -     | -     |

* Clones were analyzed with ¹¹I-labeled target cells for donor B and ¹³C-labeled target cells for donor A. Maximum release and spontaneous release, respectively, in analysis of donor A was: Kh 2,201, 321; bml8 2,397, 310; bml1 1,897, 222; bml2 1,891, 264; bml4 2,126, 309; bml9 2,566, 324; bml10 2,363, 454; bml11 2,194, 311; D2.GD 2,387, 227. In analysis of donor B it was: Kh 1,065, 246; bml8 759, 171; bml1 1,137, 262; bml2 1,294, 459; bml3 771, 160; bml4 769, 173; bml5 1,008, 216; bml10 1,973, 306.

**Panel Analysis of Monoclonal CTL.** Clones that successfully expanded as described above were tested the next day in duplicate on each of nine target cells. This panel includes seven different H-2K<sup>a</sup> mutants, C57BL/6Kh (standard strain), and D2.GD (H-2K<sup>d</sup> and H-2-D<sup>b</sup>) as a control for reactivity against an antigen other than H-2K<sup>b</sup>. The results from a typical panel analysis of clones obtained from two different donors are given in Table II. For the purpose of assigning reactivity patterns to each clone, the following criteria were used. First, the value for lysis on D2.GD was subtracted from each value obtained on other panel targets. Clones were considered only if lysis on the standard (C57BL/6Kh[Kh]) exceeded 15%. Reactivity on a mutant was judged positive if lysis was ≥60% than the value on Kh. Reactivity was considered negative if this value was ≤25%. Clones that exhibit partial reactivity (>25%, <60% of the value on Kh) on any target were considered ambiguous, or potentially the product of two precursor cells and not considered in further analysis of repertoire. The distribution of reactivity patterns for 43 clones obtained from 7 individual donors is presented in Table III.

As demonstrated by donors B and D, it was occasionally found that an individual was represented by a high proportion of a particular reactivity pattern. This could...
### Table III

Reactivity Patterns Obtained for Anti-H-2K<sup>b</sup> Clones from Individual Donors

| Reactivity target cell pattern | Donor* |
|-------------------------------|--------|
| | A | B | C | D | E | F | G |
| 1 | + | + | + | + | + | + | + |
| 2 | + | + | + | - | + | + | + |
| 3 | + | + | - | - | + | - | + |
| 4 | + | + | - | - | + | - | - |
| 5 | + | + | - | - | + | - | - |
| 6 | + | - | + | + | + | + | + |
| 7 | + | - | + | + | + | + | + |
| 8 | + | - | + | + | + | + | - |
| 9 | + | - | + | + | + | + | - |
| 10 | + | - | - | - | + | - | + |
| 11 | + | - | - | - | + | + | + |
| 12 | + | - | + | + | + | + | - |
| 13 | + | - | - | + | + | + | - |
| 14 | + | - | - | + | + | - | + |
| 15 | + | - | - | + | - | + | + |
| 16 | + | - | - | + | - | + | - |
| 17 | + | - | - | - | + | - | + |
| 18 | + | - | - | - | + | - | - |
| 19 | + | - | - | - | - | + | - |
| 20 | + | - | - | - | - | - | - |
| 21 | + | - | - | + | + | + | + |
| 22 | + | - | - | + | + | - | - |
| 23 | + | - | - | + | + | - | - |

* Numbers refer to the number of clones of each particular reactivity pattern obtained in each individual.

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### Table IV

Reactivity Patterns Observed for All Donors

| bm9 | bm10 | bm11 |
|-----|------|------|
| +   | +    | +    |
| +   | +    | +    |
| +   | +    | +    |
| +   | +    | +    |
| 2*  | +    | +    |
| +   | +    | +    |
| +   | +    | +    |
| 2   | +    | +    |
| 2   | +    | +    |

* Numbers refer to the number of different donors that demonstrate clone(s) exhibiting a particular reactivity pattern. The data consider 30 clonotypes obtained from data in Table III as described in Results.

### Table V

Distribution of Clones Amongst Reactivity Patterns

| Number of animals in which each clonotype was observed | Predicted distribution* | Actual distribution |
|-------------------------------------------------------|-------------------------|---------------------|
| 0                                                     | 101.1                   | 105                 |
| 1                                                     | 23.68                   | 16                  |
| 2                                                     | 2.78                    | 7                   |

* Represents the number of reactivity patterns expected in each category on the basis of a random distribution of 30 clonotypes amongst 128 possible reactivity patterns as determined by Poisson distribution.

Many of the outstanding questions concerning T cell specificity and genetic determination of the lymphocyte receptor repertoire could be resolved by direct repertoire analyses. For example, comparison of repertoires expressed in strains of selected genetic nonhomology, such as H-2 and allotype, would identify the influence of genetic polymorphism on expression of individual specificities. In addition, it would be possible to evaluate the degree of repertoire overlap between different lymphocyte subsets, such as T and B lymphocytes, or functionally distinct T lymphocyte subsets.
TABLE VI
Antigenic Differences Between H-2 Mutants and Standard H-2K

| Strain   | Percentage of observed clonotypes that did not include recognition of mutant* |
|----------|--------------------------------------------------------------------------------|
| bm1      | 74                                                                              |
| bm8      | 61                                                                              |
| bm3      | 52                                                                              |
| bm4      | 56                                                                              |
| bm9      | 26                                                                              |
| bm10     | 52                                                                              |
| bm11     | 43                                                                              |

* Data obtained by dividing the number of clonotypes that did not include recognition of each particular mutant by the total number of antigenic determinants described. 100% would indicate total dissimilarity in all 23 determinants present on the standard strain.

The results presented above confirm the feasibility of applying the method of panel analysis to monoclonal CTL for the purpose of dissecting the CTL receptor repertoire. It should be stressed, however, that although application of panel analysis in a manner analogous to previous studies of the B cell repertoire enables discrimination of all clones responsive to distinct determinants of a particular antigen, such analysis does not delineate among the sets of clones that give similar reactivity patterns. Further discrimination would require immunochemical analysis of the receptor molecule.

As applied to the study of the CTL receptor repertoire, the technical requirements for panel analysis include (a) the ability to obtain monoclonal CTL, (b) the means by which they may be clonally expanded, and (c) an appropriate discriminatory panel. To assure that the first criterion was met, clones were generated at low frequencies. In addition, a second precautionary criterion was imposed on the data in the assignment of reactivity patterns. Specifically, clones that did not lyse at comparable levels on different targets were not included. This criterion probably eliminated some true clones because the number of clones that showed partial reactivities was higher than indicated by the predicted frequency of two precursors in a single well. This could indicate that some antigenic determinants on the mutants were altered only to the extent that there are slight affinity differences in recognition.

As demonstrated above, clones may be expanded with partially purified rat TCGF. In some experiments, as many as 50% of the clones increased at least 10-fold in their lytic potential after 5 d of incubation in media that contained factor. It is possible that that appropriate conditions could be found to promote expansion of all clones. Although attempts have not, as yet, been made to maintain clones indefinitely, it has been found that subsequent to expansion, CTL clones may be subcloned by limiting dilution and reexpanded in media that contain TCGF. This is currently being done on selected clones to check monoclonality.

It was occasionally found that an individual donor expressed a high proportion of a particular clonotype. Because the reiterated clonotypes varied in different individuals they probably do not represent genetically determined predominant clonotypes. One likely explanation for this finding is that, as is the case for B cell precursors, CTL precursors develop clonally in each individual such that any one animal could exhibit...
multiple representation of a limited number of clonotypes. For this reason, to statistically analyze the specificity repertoire of the strain as a whole, reactivity patterns were counted only once per individual. By such an analysis it is clear that the high frequency of alloreactive T cells reflects the summation of a large number of different clonotypes that are directed against distinct determinants on the H-2 molecule. Therefore, theoretical consideration regarding the high frequency of alloreactive cells must consider the large number of distinct antigenic determinants that are recognized. The implications of this finding are difficult to reconcile with any theory that considers that alloreactive specificities represent a discrete number of evolutionarily conserved idiotypes. In this regard, these results appear contrary to the findings of Binz et al. (41, 42), which suggest a highly restricted allo-specific idiotypic repertoire. It is possible to reconcile these data by assuming that their anti-idiotypic antisera are composed of numerous specificities or that the particular strain combination used in their studies results in a more-restricted response than does B10.D2 anti-H-2K\textsuperscript{b}.

Despite the large number of different clonotypes present in the B10.D2 repertoire, the distribution of reactivity patterns observed in these analyses is more restricted than would be predicted for a sampling of a repertoire that is randomly distributed amongst 128 potential reactivity patterns. One possible explanation for this result is that not all theoretically possible patterns are actually recognizable by B10.D2 as antigenic determinants. By assuming that the distribution obtained represents a randomly assorted repertoire, it may be estimated that only \(\sim 50\) different reactivity patterns are actually possible. Although this is not the only possible explanation for the data, it provides a minimum estimate of the number of different specificities present in the B10.D2 anti-H-2K\textsuperscript{b} repertoire. Alternative explanations would consider that either the repertoire is weighted in favor of particular clonotypes (predominant clonotypes) or that certain reactivity patterns are highly favored because of increased immunogenicity. If either of these assumptions is imposed on the data, then the predicted number of specificities present in the repertoire would increase.

It is possible, therefore, to place a lower limit on the size of the B10.D2 anti-H-2K\textsuperscript{b} repertoire by assuming 50 different specificities. The experimental data indicate that the frequency of anti-K\textsuperscript{b} clones is 1 in 12,000 spleen cells (Fig. 1). Considering the Ly-2,3 T cell population represents \(\sim 3\%\) of spleen cells, it may then be calculated that the frequency of Ly-2,3 CTL precursors specific for any one determinant on H-2K\textsuperscript{b} is 1 in 18,000. If it is assumed that all specificities are comparably represented in the strain, then this suggests that the Ly-2,3 repertoire contains a minimum of 18,000 specificities. This is clearly a minimum estimate for the size of the CTL repertoire because it assumes all reactivity patterns represent a single clonotype. Although it is impossible at this time to evaluate the upper limit on the size of the CTL repertoire, it is not inconceivable that it may approach the size of the B cell repertoire. In this regard, experiments analyzing the anti-H-2K\textsuperscript{b} repertoire of B cells are currently underway to evaluate the extent of repertoire sharing between B and T cells.

The method of panel analysis not only provides information concerning repertoire, it serves to dissect the antigen as well. One rather surprising feature of the results obtained above is that the invariant portion of the H-2K\textsuperscript{b} molecule, as identified by clones reactive with all mutants, was recognized by a low proportion of the B10.D2 anti-H-2K\textsuperscript{b} repertoire. Only two of seven animals studied displayed clones of this
particular reactivity pattern. This would indicate that most of the antigenic regions of the molecule have been altered in one or more mutants. Biochemical studies should reveal if this correlates with an actual lack of an invariant H-2Kb-like region of the molecule. Perhaps even more surprising is the extent to which each mutant appears antigenically distinct from the standard H-2Kb molecule. It has been demonstrated that only slight biochemical differences exist between standard H-2Kb and several of the mutants such as B6.C-H-2bm1 and -bm8 (36, 37). However, in agreement with previous studies evaluating the antigenicity of these mutants, there are more antigenic differences than similarities between several of these mutants and the standard molecule. This probably reflects the selection pressure of the method used for detection of H-2 mutants that is based on skin graft rejection and that may require multiple antigenic differences to be effective. In this regard, the bg1 and bg2 mutations that arose independently do not reject grafts; however, preliminary evidence indicates antigenic differences do exist between these mutants that are detectable by ~15% of the anti-H-2Kb clones investigated.

The results reported above indicate the success of the method of panel analysis in the dissection of the CTL receptor repertoire directed against an alloantigen. It should now be possible to extend these analyses to address questions concerning CTL repertoire development and to evaluate the role of different genetic and environmental factors on the acquisition of particular receptor specificities. In addition, these studies have demonstrated the ability of this approach to probe the antigenic composition of a molecule. This should prove a valuable tool in our understanding of CTL fine specificity.

Summary

The B10.D2 cytolytic T lymphocyte (CTL) receptor repertoire specific for the H-2Kb alloantigen has been studied by determining the reactivity patterns of monoclonal CTL against a panel of seven different H-2Kb mutants. The repertoire is extremely diverse and contains a minimum of ~50 different specificities against unique antigenic determinants on the H-2Kb molecule. Each specificity appears at a maximum frequency of 1 in 18,000 CTL precursors. These studies have also served to dissect the antigenic composition of the H-2Kb molecule. Very few CTL clonotypes share recognition of all the mutants, thereby indicating the lack of conservation of a b-type antigenic region. In addition, the degree to which each mutant shares antigenic determinants with the standard H-2Kb molecule has been determined.

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