INFLUENCE OF THE MAJOR HISTOCOMPATIBILITY COMPLEX ON THE REPERTOIRE OF ALLOSPECIFIC CYTOLYTIC T LYMPHOCYTES*

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Many unresolved issues concerning determinative genetic and environmental contributions to immune responsiveness may best be answered by the direct examination of the lymphocyte receptor repertoire. With this goal in mind, this laboratory recently introduced an experimental approach that permits analysis of the cytolytic T lymphocyte (CTL)[1] precursor repertoire specific for the H-2K^b alloantigen (1). This entails (*a*) obtaining a large number of independently derived monoclonal H-2K^b-specific CTL by limiting dilution techniques; (*b*) expanding each clone using T cell growth factor; and (*c*) identifying the receptor specificity of each clone by fine specificity analysis using a panel of target cells derived from strains of mice that bear different mutations within the H2K^b molecule. The receptor specificity of each clone is thereby identified and distinguished on the basis of the distribution amongst the mutants of the antigenic determinant that it recognizes. Using these techniques, a preliminary analysis of the B10.D2 anti-H-2K^b specificity repertoire based on CTL clones obtained from seven individual animals led to the conclusion that the K^b-specific receptor repertoire was extremely diverse and extrapolated to a minimum of 50 unique receptor specificities (1).

This type of experimental approach is potentially of value in identifying genetic loci involved in repertoire determination. However, the ability to perform comparative genetic analysis is based on several assumptions yet to be proven: (*a*) that the CTL repertoire of an inbred strain exhibits at least some repertoire characteristics that are genetically inherited and expressed by a large proportion of the individual members of the strain, and (*b*) that the experimental technique used for repertoire analysis is capable of identifying these phenotypic characteristics. To determine whether these conditions are fulfilled, an extensive analysis of the anti-H-2K^b CTL specificity repertoire of two genetically different murine strains has been performed and is reported below.

Two congenic resistant strains of mice that differ within the major histocompatibility complex (MHC), B10.BR and B10.D2, were used in this investigation. MHC-disparate strains were chosen for such a comparative repertoire study on the basis of the well-documented influence of MHC on CTL responsiveness. MHC-encoded antigens act as restricting elements, such that expression of effector cell function is

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[1] *Abbreviations used in this paper:* CTL, cytolytic T lymphocyte(s); F_1, (B10.BR × B10.D2)F_1; Ir, immune response; MHC, major histocompatibility complex; R.P., reactivity pattern.

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dependent on recognition of H-2 antigens on target cells that are identical to those presented on stimulator cells (2, 3). The MHC also encodes immune response (Ir) genes that determine the level of CTL responsiveness against a variety of different viral-infected or chemically modified cells (3–10). In addition, studies using radiation chimeras have provided a large body of evidence that implicates the MHC at the level of functional expression of certain receptor specificities (3, 6, 10–12). Although very little is known concerning the mechanism by which H-2 antigens exert such effects, the above findings suggest that genes within the MHC may direct selection of the receptor repertoire that is permitted on CTL precursors. Therefore, MHC-disparate strains were obvious candidates for testing the feasibility of comparative repertoire studies.

The results reported below indicate that despite considerable diversity within the anti-H-2K^b specificity repertoire, a portion of the response includes specificities that recur at a comparatively high frequency within an inbred strain. It is these recurrent specificities that provide phenotypic markers for the CTL repertoire of each strain and thereby permit repertoire comparisons. The frequency of expression of such recurrent specificities was found to be very different in the B10.D2 and B10.BR strains, which indicates that one way in which the MHC may influence repertoire is by regulation of the frequency of precursors with particular receptor specificities.

To explore the characteristics of the mechanism responsible for the observed MHC-related repertoire differences and to compare these results with other immune responses that are influenced by MHC, the anti-K^b response of (B10.BR × B10.D2)F$_1$ progeny was analyzed. It is observed that the F$_1$ repertoire preferentially maintains elements of the B10.D2 but not of the B10.BR parental strain. However, most surprising was the large proportion of the F$_1$ repertoire that was devoted to specificities that are sparsely represented in either parental repertoire. These results are discussed in relationship to possible mechanisms responsible for the observed influence of MHC on the CTL specificity repertoire and also are compared with other types of MHC-regulated immune responsiveness.

Materials and Methods

Experimental Animals. All murine strains used in these studies were obtained from the Scripps Clinic and Research Foundation Breeding Colony. C57BL/6 Kh, B6.C-H-2^{bm1}, B6.H-2^{bm3}, B6.C-H-2^{bm4}, B6.H-2^{bm8}, B6.C-H-2^{bm9}, B6.C-H-2^{bm10}, B6.C-H-2^{bm11}, and D2.GD were used as a source of target cells in panel analysis. B10.A(5R) females were used as a source of stimulator cells. CTL clones were derived from 8- to 12-wk-old B10.D2, B10.BR, and (B10.BR × B10.D2)F$_1$ females. On occasion, B10.BR mice were also obtained from The Jackson Laboratory, Bar Harbor, Maine. Lewis rats used in the preparation of T cell growth factor were retired breeders purchased from Microbiological Associates, Walkersville, Md.

Repertoire Analysis. All techniques involved in the stimulation of H-2K^b-specific CTL precursors in limiting dilution cultures, detection, and expansion of the resultant CTL clones, as well as fine-specificity analysis of receptor specificity using a panel consisting of nine different target cells were as previously described (1). Stimulator cells used in limiting dilution cultures were obtained from B10.A(5R) female mice (H-2K^b,D^d). Responder cells were of the indicated strain of origin, and "helper" cells were syngeneic with responders. Stimulator cells used in clonal expansion were of either B10.A(5R) or C57BL/6 origin. T cell growth factor was prepared using rat spleen cells and used without further purification, as previously described (1, 13).

Reactivity patterns (R.P.) were assigned to each clone using the following criteria: (*a*) the value obtained for percent specific lysis on the D2.GD target used as a negative control (H-2K^d,

D^b) was subtracted from each value calculated for the eight other target cells included in the panel, C57BL/6 Kh (wild type), and seven different H-2K^b mutants. A clone was considered positive for recognition of a particular K^b mutant if lysis was ≥60% of the value obtained on the C57BL/6 Kh target that bears the stimulating antigen and was considered negative if lysis was ≤25% of this value. Clones that exhibited intermediate value of lysis (between 25 and 60%) on any one of the mutants were not assigned an R.P. and were not considered further in these analyses. Of course, individual clones varied greatly in their lytic activity, however, most clones considered in repertoire analysis exhibited ≥50% specific lysis on the H-2K^b-bearing panel target.

Approximately 5% of B10.BR clones demonstrated equivalent levels of lysis on D2.GD and C57BL/6Kh target cells, yet did not recognize all of the K^b mutants. Such clones demonstrate recognition of both H-2K^b and H-2K^d. These specificities were not included in the B10.BR anti-H-2K^b repertoire.

Reactivity Patterns. To facilitate discussion, each of the 128 possible combinations of positive and negative reactivity on the panel of seven different K^b mutants has been assigned an R.P. number, as indicated in Table I.

Results

The B10.D2 Anti-H-2K^b CTL Specificity Repertoire. Considering the great diversity of the allospecific CTL receptor repertoire (1) and the low frequency of representation of any one receptor specificity, it is only possible to consider a particular specificity for the purposes of repertoire comparison if it is present at a frequency much greater than would be predicted on the basis of random distribution. It was, therefore, necessary to extend the previous examination of the B10.D2 repertoire to determine whether recurrent specificities exist within the CTL specificity repertoire.

Fig. 1 A describes the distribution of clonal specificities obtained for 78 clones derived from 20 individual B10.D2 mice. The data is presented as both the number of animals in which clones of a particular R.P. were observed and as the fraction of

TABLE I
Reactivity Patterns

				bm9	+	+	+	+	−	−	−	−
				bm10	+	+	−	−	+	+	−	−
				bm11	+	−	−	+	+	−	−	+
bm8	bm1	bm3	bm4									
+	+	+	+		1*	17	33	49	65	81	97	113
+	+	−	+		2	18	34	50	66	32	98	114
+	−	−	+		3	19	35	51	67	83	99	115
+	−	+	+		4	20	36	52	68	84	100	116
−	+	+	+		5	21	37	53	69	85	101	117
−	+	−	+		6	22	38	54	70	86	102	118
−	−	−	+		7	23	39	55	71	87	103	119
−	−	+	+		8	24	40	56	72	88	104	120
+	+	+	−		9	25	41	57	73	89	105	121
+	+	−	−		10	26	42	58	74	90	106	122
+	−	−	−		11	27	43	59	75	91	107	123
+	−	+	−		12	28	44	60	76	92	108	124
−	+	+	−		13	29	45	61	77	93	109	125
−	+	−	−		14	30	46	62	78	94	110	126
−	−	−	−		15	31	47	63	79	95	111	127
−	−	+	−		16	32	48	64	80	96	112	128

* Numbers have been arbitrarily assigned to each R.P. for purposes of identification and discussion.

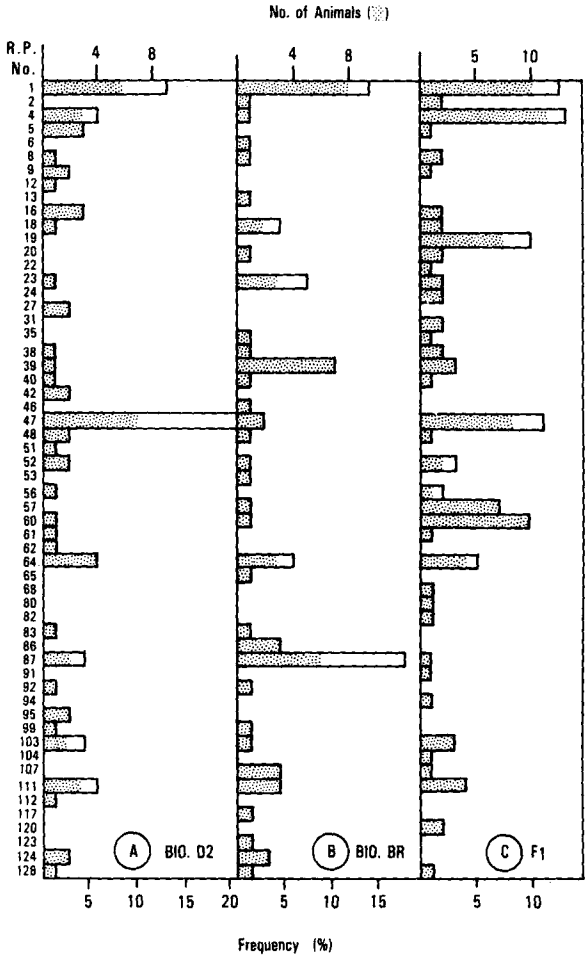

FIG. 1. Anti-H-2K^b CTL receptor repertoire of B10.D2, B10.BR, and F_1 progeny. The specificity referred to by each R.P. number is given in Table I. Only those R.P. that are representative of clones observed in these analyses are listed. Number of animals refers to the number of individual mice in which each R.P. was observed. The frequency of clones that exhibit R.P. 1 was calculated by dividing the number of clones that exhibited R.P. 1 by the total number of clones included in each repertoire analysis. The frequency of all other R.P. was calculated in an analogous manner except that the total number of clones did not include clones that exhibited R.P. 1 as discussed under Results.

the repertoire that is described by clones of that particular R.P. 33 different receptor specificities were identified in these analyses. For the purpose of further discussion, clones are referred to by their designated R.P. R.P. 1 represents clones that recognize all of the mutants included in the panel of target cells. Considering that the criteria for recognition of H-2K^b is recognition of an antigen that is differentially represented on the H-2 mutants, clones expressing R.P. 1 may, in theory, recognize a molecule other than H-2K^b and are therefore omitted from further consideration in this particular analysis.

If the remaining clones are analyzed by Poisson distribution, it is impossible to reconcile the frequency of recurrence of R.P. 47 with a random distribution of receptor

specificities. R.P. 47 appeared in over one-third of the individuals studied and represents the specificity of 20% of all anti-H-2K^b-specific clones that are found in B10.D2 mice. Based on the number of antigen-specific clones (69) and an estimated repertoire size of 50 unique receptor specificities, Poisson statistical analysis would predict a <0.01 probability that a randomly distributed specificity would represent >7% of the repertoire. Considering that R.P. 47 represents 20% of the repertoire, this specificity is clearly nonrandomly distributed and may, therefore, be considered a distinguishing repertoire characteristic.

The B10.BR Anti-H-2K^b CTL Specificity Repertoire. The existence of a highly recurrent specificity that could serve as a repertoire probe encouraged the prospect of performing comparative analysis between strains of defined genetic nonhomology. Therefore, the repertoire of the congenic strain B10.BR (H-2^k) was investigated next. The frequency of B10.BR anti-H-2K^b-specific CTL precursors is comparable to that of B10.D2 (data not shown). The distribution of specificities obtained from 17 B10.BR individuals is described in Fig. 1 B. Similar to B10.D2, the repertoire of B10.BR anti-H-2K^b receptor specificities is highly diverse. In this case, the 78 clones that were analyzed describe a total of 34 different receptor specificities. It should be stressed that although the R.P. of many B10.BR clones are indistinguishable from those of B10.D2, it cannot be concluded that clones with identical R.P. bear identical receptor idiotypes.

Omitting further consideration of R.P. 1, three specificities, R.P. 23, 39, and 87, recur at a relatively high frequency, 7.4, 10.4, and 17.9%, respectively. Two of these were expressed by over one-third of individuals. Most significant, however, for the purpose of repertoire comparison is the fact that the B10.BR recurrent specificities do not predominate in the B10.D2 repertoire; neither does R.P. 47, which was highly recurrent in B10.D2, predominate in the B10.BR repertoire. Thus, clones expressing R.P. that are clearly diagnostic of the two repertoires in question differ significantly in their frequency of recurrence in two strains that are genetically identical in all but the MHC locus.

The Specificity Repertoire in (B10.BR × B10.D2)F_1 Progeny. To explore the basis for MHC regulation of the expression of recurrent specificities, the anti-H-2K^b CTL receptor repertoire was examined using F_1 progeny. Fig. 1 C presents the results obtained in an examination of 121 clones obtained from 26 individuals. The response was similar to that of the parental strains with respect to the frequency of K^b-specific CTL precursors and in the diversity of the receptor repertoire. These 121 clones described 38 different receptor specificities.

Of those R.P. that represent recurrent parental specificities, only R.P. 47, which is characteristic of the B10.D2 parental strain, appears as a recurrent specificity within the F_1 repertoire. However, four other receptor specificities (R.P. 4, 19, 57, and 60) emerge as uniquely F_1 recurrent specificities.

Discussion

The experiments described above explore the feasibility of using specificity analysis of monoclonal CTL as a vehicle for comparative repertoire studies between inbred strains of defined genetic nonhomology. The validity of this experimental approach is dependent on the existence and successful identification of repertoire markers that may serve as strain-specific phenotypic characteristics. The results reported herein

describe several nonrandomly represented, recurrent specificities that serve as markers and thereby permit comparative analysis of the CTL receptor repertoire.

Considering that each R.P. may describe one or more antigenic determinants, each of which may be recognized by one or more receptor idiotypes, the basis of R.P. recurrence is uncertain. A recurrent specificity may be similar to previously described repertoire markers, such as predominant idiotypes that have been used in the analysis of genes that encode and/or regulate both B and T cell idiotypes (14–16). Alternatively, a recurrent specificity may represent enhanced recognition of a particular MHC determinant accomplished by idiotypically unrelated CTL receptors. Regardless of the idiotypic constitution of the receptors represented by a recurrent R.P., their genetic heritability permits their exploitation as a repertoire marker.

Although defined on the basis of frequency, each recurrent specificity was observed in a large proportion of individuals within a strain. Considering that only a small and variable fraction of the repertoire in each individual was sampled, lack of detection of a particular specificity in cells derived from an individual does not signify its absence but rather suggests an upper limit for its frequency of representation. Therefore, these results are consistent with the interpretation that the repertoire of each individual reflects the repertoire of its strain of origin. However, a more extensive analysis of the repertoire present within each individual would be necessary to verify this conclusion.

Comparison of the H-2K^b-specific CTL repertoire of two congenic strains of mice that differ specifically within their MHC indicates a profound influence of MHC-linked genes on the frequency of representation of several receptor specificities. For the purpose of comparison with previously described analyses of the mechanism of MHC-linked regulation, it is important to point out several unique features of the experimental system used in obtaining the CTL clones described herein. First, there is a minimum selective role for either helper or suppressor T cells in contributing to responsiveness. Help is provided identically to all CTL precursors in the form of allogeneically induced T cell growth factors produced by the interaction of large numbers of syngeneic helper spleen cells and antigen-bearing stimulator spleen cells. In addition, the potential influence of any suppressor cells that might have been present in these cultures appears to have been minimized by limiting dilution, as evidenced by the linearity of the response with increasing numbers of responder cells (1). Second, unlike many other experimental systems in which antigen responsiveness is subject to regulation by MHC-linked Ir genes, because syngeneic antigen-presenting cells do not appear to be involved in presentation of alloantigens to CTL precursors (17), cellular interaction probably does not regulate responsiveness in this system. For these reasons, the observed influenced of MHC on the expressed CTL repertoire presumably reflects differences in the actual frequencies of precursors that bear particular receptor specificities rather than differential regulation attributable to help, suppression, or antigen presentation (6, 18–21).

Potentially, there are a variety of ways in which the MHC could lead to differences within the CTL specificity repertoire. Of course, one interpretation is that MHC-encoded genes physically contribute to the T cell receptor. This would be consistent with other experimental results that suggest the MHC influences the idiotypic repertoire of alloreactive T cells (22). However, many of the ways in which the MHC effects the T cell repertoire may also be explained as regulatory functions. For

example, MHC determination of restriction specificity may serve as a positive regulatory influence during repertoire selection (3), whereas tolerance to autologous MHC-encoded antigens may serve as a negative influence on the receptor repertoire available to T cell precursors (23). Either or both of these mechanisms may contribute to MHC influence on the allospecific CTL receptor repertoire (12). Consideration of the constraints imposed by the experimental results suggest it is unlikely that the repertoire differences reported above are solely attributable to a simple deletional tolerance mechanism. Considering the level of shared CTL determinants (24, 25), it would be anticipated that no more than 5–10% of anti-K^b CTL clones would cross-react with a third-party alloantigen. Therefore, it is unlikely that all three B10.BR recurrent specificities, which together comprise over one-third of the anti-K^b response, are eliminated from the B10.D2 repertoire because of the presence of H-2^d antigens.

To explore further the mechanism responsible for the observed repertoire differences between B10.D2 and B10.BR, the response of F_1 progeny was analyzed. The F_1 repertoire maintained frequent representation of the B10.D2 recurrent specificity but not any of the B10.BR recurrent specificities. One interpretation of this result is genetic recessiveness of B10.BR repertoire expression. Alternatively, in consideration of the limited number of recurrent specificities that were available for analysis, these results might reflect the independent regulation of individual specificities regardless of their parental origin. Whatever the mechanism responsible for expression of the B10.D2 recurrent specificity, its maintenance in the F_1 environment further argues against tolerance as the cause for its relatively low level of expression within the B10.BR parental repertoire. In addition, recent analysis of the repertoire in neonatal F_1 progeny reveals the expression of recurrent specificities from both parental strains (manuscript in preparation).

Perhaps the most surprising feature of the F_1 repertoire is the expression of four recurrent specificities that do not predominate in either parental repertoire. Analogous results have been obtained in studies of the B cell specificity repertoire expressed by F_1 hybrids (26). Although this probably reflects the resolution of many levels of repertoire regulation, it is interesting to speculate on a possible relationship between the appearance of distinguishing F_1 specificities and the formation of unique F_1 determinants via combinatorial association of I-region-encoded antigens (27). Future experiments designed to identify the genetic locus within the MHC that is responsible for differential expression of recurrent specificities should help to determine the relevance of this analogy. Although previously described MHC-linked Ir genes that affect CTL responsiveness have more commonly been mapped to the H-2K or D region rather than the I region, one type of responder phenotype in which the specificity rather than the magnitude of a CTL response is subject to MHC-linked regulation has recently been localized to the I region (28).

In conclusion, the experiments described herein demonstrate the heritability of at least a portion of the CTL specificity repertoire. Each strain exhibited a receptor repertoire of sufficient diversity as to preclude assessment regarding an individual's representation of most specificities; nevertheless, the repertoire markers obtained in these studies were sufficient to permit meaningful comparative genetic analysis of the allospecific CTL repertoire. It is anticipated that these same recurrent specificities will serve as phenotypic markers in future repertoire analyses in which the contribution of other genetic loci, including immunoglobulin-linked allotype genes, is evaluated.

In addition, it is hoped that techniques similar to those used above may be used in the future to analyze the receptor repertoire specific for conventional antigens. However, in view of the increasing evidence that suggests overlap between the antigen-specific and allospecific T cell receptor repertoires, it is anticipated that much of the information obtained in the course of the analyses of an allospecific repertoire will pertain equally well to the antigen-specific repertoire (29–32).

Summary

Superimposed on the heterogeneous anti-H-2K^b cytolytic T lymphocyte (CTL) receptor repertoire of allogeneic murine strains are reactivities that recur with high frequency amongst individuals of any given strain. These receptor specificities represent phenotypic markers of the CTL repertoire and, as such, have been used to compare receptor repertoires of genetically disparate strains. The results demonstrate that congenic strains differing only in the MHC (B10.D2 and B10.BR) differ significantly in their H-2K^b-specific CTL repertoires. This finding clearly demonstrates a role for the MHC in determination of the CTL precursor repertoire.

The mechanism by which MHC influences CTL specificity was explored through analysis of the anti-H-2K^b repertoire of (B10.BR × B10.D2)F_1 hybrids. Because at least one recurrent parental specificity has found to be recurrent in F_1 progeny as well, the findings indicate that MHC-specific tolerance cannot be solely responsible for repertoire differences between MHC-disparate strains. In addition, the F_1 repertoire is characterized by the emergence of several nonparental recurrent specificities.

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