Brief Definitive Report

GENETIC LINKAGE OF THE CYTOLYTIC T LYMPHOCYTE REPERTOIRE AND IMMUNOGLOBULIN HEAVY CHAIN GENES*

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A variety of experimental approaches have been used to study genetic determination of the T cell receptor. Evidence that anti-idiotypic antibodies prepared against immunoglobulin bind T cells or T cell products that demonstrate the appropriate antigen specificity suggests genetic linkage between T and B cell idiotypes (1–5). In contrast, T lymphocytes that demonstrate a requirement for recognition of molecules encoded by the major histocompatibility complex (MHC) as well as foreign antigen, such as proliferative or cytolytic T lymphocytes (CTL), do not appear to express idiotypes that predominate in antibody responses (4, 6–8). An alternative strategy that has been successfully applied to the study of genetic control of allospecific T cell receptors has been to obtain anti-idiotypic antisera by direct immunization with allospecific T cells (2, 4). Using such antisera, Krammer and Eichmann (9) demonstrated that both the MHC and immunoglobulin heavy chain genes (Igh) participate in genetic determination of murine T cell idiotypes. In a similar study in the rat, Binz and co-workers (10) found that idiotype expression was linked to Igh but not to MHC. Also in the rat, but using presumptive anti-receptor T cells rather than anti-receptor antibodies, Bellgrau and Wilson (11) concluded that allospecific T cell receptors displayed little if any polymorphism and thus found no evidence for linkage to polymorphic loci such as MHC or Igh. In view of the difficulties reported in obtaining and characterizing anti-receptor reagents (6–11) as well as the ambiguities inherent in such an indirect approach (12, 13), a direct method was sought that could be applied to study genetic determination of the T cell repertoire.

Recent advances in techniques of T cell cloning have permitted analysis of the CTL receptor repertoire by studying the fine specificity of monoclonal CTL. As previously reported (14), this approach has supplied a detailed description of the $H\text{-}2K^b$-specific receptor repertoire by identifying and distinguishing each of a large number of $H\text{-}2K^b$-specific clones on the basis of their reactivity against a panel of $H\text{-}2K^b$ mutants. This has proven to be a useful alternative to anti-idiotypic antisera for studying genetic control of the T cell receptor repertoire. In view of the great diversity of receptor specificities within the $H\text{-}2K^b$-specific repertoire, the success of repertoire analysis as a tool for genetic studies is attributable to the existence, within a given inbred strain, of one or several receptor specificities that recur at an unusually high frequency. In a previous study (15, 16) that compared the specificity repertoire of two MHC congenic strains, B10.D2 and B10.BR, it was found that each strain expressed different recurrent specificities. Therefore, recurrent specificities are useful phenotypic markers. In studies designed to probe the mechanism responsible for the

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effect of MHC on repertoire, it was observed (17) that MHC regulation increased during development, insofar as 10–14-d-old neonates representing these same MHC-disparate strains expressed several repertoire similarities that were not observed in their adult counterparts.

In this report, the strategy of direct analysis of the monoclonal H-2K^b-specific CTL repertoire has been used to evaluate the contribution of polymorphic loci other than MHC by comparing the CTL repertoire of two strains, BALB/c and B10.D2, that share the H-2^d haplotype, yet whose genetic backgrounds are presumably of independent origin. In addition, the contribution of Igh-linked genes has been assessed by analyzing the CTL repertoire expressed by the allotype congenic strain CB-20 that shares the Igh^b haplotype with B10.D2 but has the BALB/c background. This has permitted an evaluation of the role of Igh-linked genes and the background (non-H-2, non-Igh) genetic complement in expression of the CTL receptor repertoire. The findings indicate that the only genetic loci that differ between BALB/c and B10.D2 and affect the CTL repertoire link to Igh.

Materials and Methods

Experimental Animals. All murine strains used in these studies were obtained from the breeding colony of Scripps Clinic and Research Foundation. These include BALB/c, CB-20, B10.A(5R), D2.GD, C57BL/6Kh, and the H-2K^b mutants, 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}, and B6.C-H-2^{bm11}. Animals used as a source of responder lymphocytes were killed between 8 and 16 wk of age.

Repertoire Analysis. All techniques involved in the stimulation of H-2K^b-specific CTL precursors in limiting dilution cultures, detection of CTL clones using an H-2^b T lymphoma, EL-4, expansion of resultant CTL clones, and fine specificity analysis of receptor specificity using a panel consisting of concanavalin A-induced T cell targets from seven different K^b mutants, D2.GD (H-2K^d, D^b) and the wild type C67BL/6Kh target, are as previously described (14, 15). Stimulator cells were irradiated B10.A(5R) spleen cells. Responder cells were of the indicated strain of origin, and "helper" cells were syngeneic with responders.

Reactivity patterns (RP) 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 when lysis was $\geq$ 60% of the value obtained on the C57BL/6 Kh target that bears the stimulating antigen and was considered negative when lysis was $\leq$ 25% of this value. Clones that exhibited intermediate values of lysis (between 25 and 60%) for any one of the mutants were not assigned an RP and were not considered further in these analyses. Individual clones varied greatly in their lytic activity; however, most clones considered in repertoire analysis exhibited $\geq$50% specific lysis on the H-2K^b-bearing panel target.

Correlation Ratio. To quantitate the degree of similarity between two different repertoires, we compared strains on the basis of their expression of a set of recurrent specificities that are characteristic of one of the strains in question (17). For example, if strain A devotes X_a% of its repertoire to expression of a set of N_a different recurrent specificities and strain B devotes X_b% of its repertoire to the same set of N_a specificities, then the correlation ratio that compares strains A and B with respect to their expression of recurrent specificities characteristic of strain A is $X_b - N_aY_b/X_a - N_aY_a$, where Y represents the average frequency of representation for a single specificity in any given strain.

Results

Fig. 1 b presents the distribution of clonotypes obtained from the analysis of 117 independently derived BALB/c (H-2^d) CTL clones stimulated using B10.A(5R) (H-2K^b, D^d) cells. To assure that only clones that are indeed specific for the H-2K^b molecule are considered, the 14 clones that do not demonstrate differential

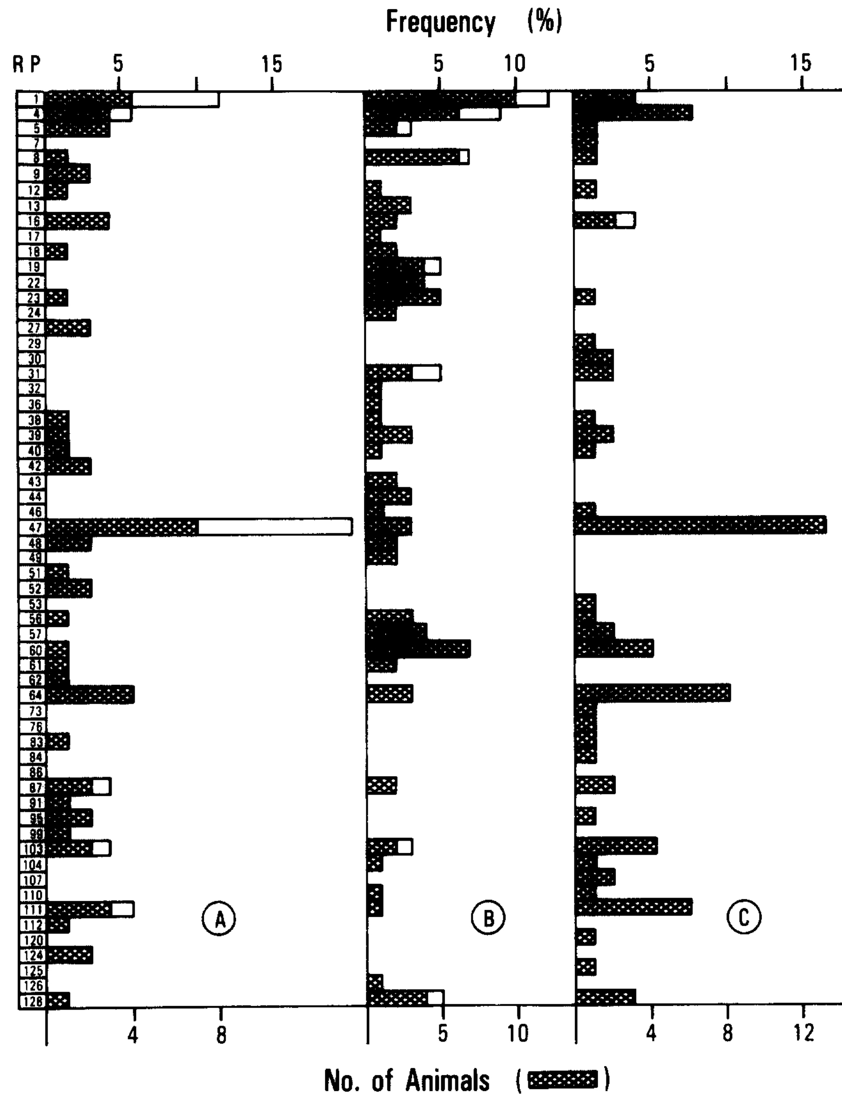

FIG. 1. Anti-H-2K^b CTL specificity repertoire. The frequency of clones that exhibit RP 1 was calculated by dividing the number of clones that exhibited RP 1 by the total number of clones included in each repertoire analysis. The frequency of all other RP was calculated in an analogous manner except that the total number of clones did not include clones which exhibited RP 1 as discussed under Results. The specificity referred to by each RP number is given in references 15 and 17. Only those RP that are representative of clones observed in these analyses are listed. Number of animals refers to the number of individual mice in which each RP was observed. A, B10.D2; B, BALB/c; C, CB-20.

recognition among the panel of K^b mutants (RP1) are not considered further in these analyses. The remaining 103 clones represent 36 different receptor specificities. Similar to the repertoire of H-2K^b specificities observed in other strains (15, and Fig. 1 b), the distribution of BALB/c clonotypes can be described as a random distribution upon which are superimposed several unusually recurrent specificities (RP 4, 8, and 60). As determined by Poisson analysis, the random component of this distribution is in good agreement with the expectation of a repertoire consisting of 38 distinct specificities.

TABLE I
*Comparative Frequencies of Recurrent Specificities**

RP number	B10.D2	BALB/c	CB-20
4	5.8	8.7	7.6
8	1.4	6.8	1.3
47	20.0	2.9	16.5
60	1.4	6.8	5.1
64	5.8	2.9	10.1
111	5.8	1.0	7.6

* Recurrent specificities refer to all specificities whose frequency of recurrence is ≥6.8% within the BALB/c repertoire, ≥7.2% within the B10.D2 repertoire, and ≥7.6% within the CB-20 repertoire. In each case the probability of random recurrence at that frequency is <0.02.

This is in contrast to the size of the B10.D2 repertoire (Fig. 1 a) that extrapolates to 47 specificities. It is of interest that, excluding recurrent specificities, all but 5 of the estimated 38 randomly distributed clonotypes have been observed.

The most pronounced disparity between the B10.D2 and BALB/c repertoires is the difference in their level of expression of RP 47. This specificity represents 20% of the B10.D2 repertoire, yet is only 2.8% of the BALB/c repertoire. Considering other specificities that are relatively frequent within the B10.D2 repertoire (RP 4, 64, and 111), only RP 4 is recurrent within the BALB/c repertoire. Conversely, RP 8 and 60 are both recurrent in BALB/c but not in B10.D2.

Among the numerous polymorphic genetic loci that differ between B10.D2 and BALB/c are Igh. To evaluate the relative contribution of Igh-linked genes to the CTL repertoire, the allotype congenic line CB-20 was investigated. This strain possesses the BALB/c background yet shares the Igh^b allelle with B10.D2. Fig. 1 c presents the distribution obtained for 79 independently derived CB-20 anti-H-$2K^b$ clones. In this case, the random portion of the repertoire extrapolates to 47 specificities, and the recurrent clonotypes are represented by RP 4, 47, 64, and 111. For the purpose of comparison, the frequency of recurrent specificities from B10.D2 (Igh^b), BALB/c (Igh^a), and CB-20 (Igh^b) are listed in Table I. It may be seen that each of the recurrent specificities characteristic of CB-20 is expressed at a comparable level within the B10.D2 repertoire. Most striking is the exceptionally recurrent expression of RP 47 in both strains that share Igh^b.

In a previous report (17), the concept of a "correlation ratio" was introduced as a measure of the degree of similarity between two repertoires based on their frequency of expression of a characteristic set of recurrent specificities. This ratio varies between 0.0 and 1.0, where 1.0 represents identity. To compare the B10.D2 and BALB/c repertoires with CB-20, the set of specificities characteristic of CB-20 (RP 4, 47, 64, and 111) are used as the basis of comparison. The correlation ratio calculated for each repertoire is as follows: CB-20, 1.0; B10.D2, 0.88; and BALB/c, 0.13. It may be concluded that B10.D2 but not BALB/c is very similar to CB-20 with respect to expression of these several recurrent specificities.

Discussion

Results obtained previously in this same experimental system demonstrated that the MHC has a profound effect on selection of the CTL receptor repertoire (15). To evaluate the contribution of polymorphic genetic loci other than MHC to the

allospecific CTL repertoire, it was necessary to compare strains that are MHC identical. Because C57BL and BALB/c are presumably of independent origin, B10.D2 and BALB/c were chosen for these studies. As discussed in detail elsewhere (15), the H-2K^b-specific repertoire of allogeneic strains is sufficiently diverse as to preclude the possibility of meaningful comparison based on the presence or absence of individual clonotypes that are randomly represented within the repertoire. Fortunately, each strain investigated thus far has exhibited at least one, and often several, clonotypes that recur at an unusually high frequency and are therefore useful as phenotypic markers for the purpose of repertoire comparison.

Comparisons of both the frequency of representation of clonotypes that have been identified as recurrent specificities and the general distributions observed reveal little similarity between the B10.D2 and BALB/c repertoire. Thus, it is clear that sharing of MHC is not sufficient to ensure T cell repertoire identity, indicating that at least one other polymorphic genetic locus must be involved in determining the T cell repertoire. In striking contrast, CB-20 is essentially identical to B10.D2 with respect to both repertoire distribution and the frequency of several recurrent specificities. Considering that CB-20 differs from BALB/c only insofar as it bears the Igh^b allele that is also present in B10.D2, these results indicate the genetic loci responsible for the differences between the B10.D2 and BALB/c repertoire are linked to Igh. These findings demonstrate a significant role for Igh-linked genes in determination of the T cell repertoire and further imply that this is the only genetic difference between these particular MHC syngeneic strains that is of consequence in determining the CTL receptor repertoire. However, this does not rule out a potential role for genetic loci that are invariant between these strains.

Although one interpretation of these results is that the structural gene for the T cell receptor is linked to Igh, in view of the capacity for CTL recognition of allotype (18), an alternative explanation for the observed Igh-linked repertoire differences could be either a positive or negative environmental selection mechanism that reflects such recognition. Future experiments will attempt to determine whether the influence of Igh-linked genes is intracellular or environmental in origin.

The results presented above concur with previous studies (9) that have demonstrated genetic linkage between expression of antigen-combining sites on T cell products and Igh and extend these findings to murine CTL. In this report, results have been obtained using a direct method for studying repertoire that is independent of and complementary to the use of anti-idiotypic antisera. A very definite advantage of this approach is that there is no ambiguity as to the relationship between the phenotypic marker under investigation and the receptor variable region. However, specificity analysis alone does not provide information concerning the relationship between T cell receptors expressed by different T cell clones or different lymphocyte subsets. Although experiments are currently in progress that will attempt to map the T cell repertoire to Igh-V- or Igh-C-linked genes, ultimately, resolution of the precise relationship between T and B cell receptors will require information at the molecular level.

Summary

The specificity repertoire of H-2K^b-specific cytolytic T lymphocytes (CTL) has been examined in B10.D2, BALB/c, and the allotype congenic line CB-20. Comparing their expression of recurrent specificities that serve as markers for the repertoire of

each strain indicates that the CTL repertoire of B10.D2 (Igh^b) and BALB/c (Igh^a) differ extensively. In contrast, the repertoires expressed by B10.D2 and CB-20 (Igh^b) are essentially identical with respect to their expression of the same recurrent specificities. Taken together with results previously obtained, it is concluded that both major histocompatibility complex and Igh-linked genes affect the CTL specificity repertoire.

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