THE FREQUENCY OF CLONES OF CYTOTOXIC LYMPHOCYTES GENERATED BY H-2 ANTIGENS*

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With the development of microculture methods, it has been possible to use limiting dilution assays to measure the frequency of precursors of cytotoxic lymphocytes (CL) (1). The results from these experiments are in agreement with less direct approaches (2-4) in establishing that a very high frequency of lymphocytes possess a specificity for a given haplotype. The investigation of CL responses against modified cell surfaces (5), virally altered cells (6), and minor H antigens (7) has revealed the central role that major histocompatibility complex (MHC) antigens play in the recognition processes involved in T-cell responses. As allogeneic combination of stimulator and responder cells differ with respect to MHC antigens and many minor non-H-2 antigens, it is of interest to establish the frequency of CL precursors that are generated in responses which are stimulated by H-2 antigens alone.

To this end a combination of cells from congenic strains of mice have been used to measure the frequency of anti-P815 (H-2d) CL. Such frequency measurements have been compared with the results derived from totally allogeneic combinations. The experiments indicate that, in the absence of non-H-2 differences, approximately 1/300 of the total number of anti-H-2d clones are stimulated.

Materials and Methods

Mice. CBA/J and DBA/2 mice are maintained as an inbred colony in the Department of Cell Biology, University of Auckland. B10.Br, B10.D2, and the appropriate F1 crosses were derived from the colony at the Walter and Eliza Hall Institute, Melbourne, Australia.

Culture System. Spleen cells were cultured in 10% fetal calf serum in RPMI-1640 (Grand Island Biological Co., Grand Island, N.Y.) with 2-mercaptoethanol to a final concentration of 5 × 10⁻⁴ M. Individual clones were segregated in a polyacrylamide raft as described previously (1). 1.3 × 10⁷ F1 stimulator cells were cultured with different numbers of parental strain responding cells.

Cytotoxicity Assay. Cells from individual dimples of the raft were removed and cytotoxicity measured by incubating with 10⁴ ⁵¹Cr-labeled P815 cells as described previously (1). Individual dimples were scored as positive when they contained greater than 10% specific lysis. The precursor frequency was calculated as described previously (1).

Results

All cultures contained 1.3 × 10⁷ F1 stimulator cells and groups of cultures were set up with either 2, 5, or 8 × 10⁶ responder cells. The number of clones produced by B10.Br spleen cells when stimulated by (B10.Br × B10.D2)F1 was

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The number of clones of cytotoxic lymphocytes in various culture combinations. The number of dimples containing cytotoxic lymphocytes are plotted against the number of responder cells in culture. B10.Br-1 were B10.Br spleen cells stimulated with (B10.Br × B10.D2)F1, B10.Br-2 were cultures of B10.Br cells stimulated with (CBA × DBA/2)F1, and CBA cultures were stimulated with (B10.Br × B10.D2)F1 cells.

![Graph showing the number of clones of cytotoxic lymphocytes in various culture combinations.](image)

**Fig. 1.** The number of clones of cytotoxic lymphocytes in various culture combinations. The number of dimples containing cytotoxic lymphocytes are plotted against the number of responder cells in culture. B10.Br-1 were B10.Br spleen cells stimulated with (B10.Br × B10.D2)F1, B10.Br-2 were cultures of B10.Br cells stimulated with (CBA × DBA/2)F1, and CBA cultures were stimulated with (B10.Br × B10.D2)F1 cells.

**Table I**

| Responder | Derived from the data in fig. 1 | Frequency of clones against P815 |
|-----------|---------------------------------|----------------------------------|
| B10.Br    | (B10.Br × B10.D2)F1             | 2.5 × 10⁻⁴                      |
| B10.Br    | (CBA × DBA/2)F1                 | 1.5 × 10⁻⁴                      |
| CBA       | (B10.Br × B10.D2)F1             | 10⁻⁴                            |
| *CBA      | (CBA × DBA/2)F1                 | 5 × 10⁻⁴                        |

* Data included from Skinner and Marbrook (1).

compared with the number generated when stimulated with (CBA × DBA/2)F1 cells by using P815 cells as targets. The ability of (B10.Br × B10.D2)F1 cells to stimulate CBA cells was also assessed and the data are expressed in Fig. 1 according to the method of Quintans and Lefkovits (8). The frequency of precursors which are detected in these cultures has been derived from these data and are summarized in Table I.

In cultures in which stimulators and responders differed solely in the H-2 region, the frequency of clones which lyse P815 was at least 300-fold lower than in cultures of cells which differed in H-2 and non-H-2 surface antigens.

**Discussion**

From a consideration of the frequency of alloreactive cells in a normal population of T cells and the number of different haplotypes, the specificity of cytotoxic responses has been described as presenting a dilemma to those attempting an analysis of T-cell recognition (9). The clonal analysis has allowed the direct estimation of the frequency of specific alloreactive precursors (1) and the same approach has provided some information about the repertoire of the cytotoxic T-cell system (10, 11).
It is generally assumed that the specificity of CL precursors is similar to the specificity of the effector cells which is an extension of the accurate sample hypothesis of Mitchison (12). If the mature CL precursors are precommitted, it follows that the larger the number of immunogenic determinants on the stimulating cell, the larger the number of clones which will be produced during an immune response. It has been found by using P815 (H-2d) as targets that B10.Br (H-2k) spleen cells generate clones of CL with a frequency of 1.5 x 10^-4 when (CBA x DBA/2)F1 cells are used as stimulators. These data demonstrate that B10.Br spleen cells contain a relatively high frequency of precursors which can produce CL able to lyse H-2d target of DBA/2 origin. This frequency is similar to the frequency of precursors in CBA mice when stimulated with either (CBA x DBA/2)F1 or (B10.Br x B10.D2)F1 cells (Table I). The interesting finding is that only 1/300 of the full anti-H-2d potential is realized when cells from the F1 of the congenic pair of mice, (B10.Br x B10.D2)F1, are used as stimulators of B10.Br cells.

The apparent inability of cells with H-2d antigens on the surface to stimulate a vigorous anti-H-2d response in congenic strain combinations points to the significance of non-H-2 antigens in such activation steps, particularly as the (B10.Br x B10.D2)F1 cells are able to stimulate a large number of precursors from CBA mice. One interpretation could be that presence of foreign non-H-2 antigens increase the immunogenicity of H-2 antigens. A number of mechanisms have been suggested that could account for such an increase such as the formation of hybrid molecules on cells (13), the involvement of multiple cell populations (14), or the existence of two receptors on precursor cells, one for H-2 (anti-H), one for non-H-2 (anti-X) (15).

There is little available information on the degree to which the non-H-2 antigens of the DBA/2 strain cross-react with the antigens controlled by the B10 background and Bevan (16) has stressed the degree to which cross-reactivity can be used to explain the high frequency of alloaggressive precursors. If these data are interpreted in terms of CL recognizing H-2 antigens in combination with a battery of minor antigens, the frequency values would suggest that there might be a large degree of cross-reactivity between the non-H-2 antigens of the CBA and B10 strains. Presumably this could be tested by assaying individual clones against more than one target as has been carried out in other types of responses (10).

Although these data have not resolved the major problem of the basis for T-cell recognition, they can be explained adequately in terms of dual receptors if the parameter of affinity is considered. One such interpretation might be that anti-H receptors are clonally distributed with a range of affinities extending from low to high levels. If the activation of precursors is related to the affinity of the receptor for the stimulating antigen (15), the 0.3% of the anti-P815 clones which are activated by the H-2 antigen alone could correspond to the high affinity precursors. These will not require dual recognition to be activated as suggested by Janeway et al. (15), and binding by the anti-X receptor is in this case irrelevant. On the other hand, low affinity anti-H-2d clones will not be activated by H-2d alone. When confronted by stimulator cells which, in addition to H-2d, have non-H-2 differences, the avidity of the precursor cells for these stimulators will be increased by additional binding via the anti-X receptor of
non-H-2 antigens. This will cause the activation of the low affinity anti-H-2\(^d\) clones and thus lead to the increased frequency as noted in Table I.

These data indicate that the clonal analysis allows a quantitative study of CL responses which, with the investigation of the specificity of individual clones, should contribute to excluding some of the current models of recognition.

Summary

By using a culture system that allows the segregation of individual precursors of cytotoxic lymphocytes, the number of clones generated by cells from different combinations of congenic mice have been measured. It has been found that 0.3\% of the total anti-H-2\(^d\) clones are generated by stimulators which differ predominantly at the H-2 locus. The contribution of non-H-2 antigens to anti-H-2 responses is discussed.

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