In vivo ANALYSIS OF CYTOTOXIC LYMPHOCYTE ACTIVITY AGAINST PRODUCTS OF THE H-2K^b GENE AND ITS MUTANT ALLELES

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There are at least two cell clones of cytotoxic T lymphocytes (CTL) which react *in vitro* against H-2 antigens. One clone acts against the product of the K clone, the other against the product of the D clone of the H-2 complex [7]. These clones can be separated by immunoadsorption on a suitable cell monolayer.

The question of whether CTL responsible for the reaction against one molecule of H-2K antigen are represented by one or several clones has not yet been finally settled. A powerful weapon for the investigation of this problem is provided by mutations of genes of the H-2 complex and, in particular, of the H-2K gene. By the use of mutants antigenic products of minimally differing alleles and the activity of CTL directed against these products can be compared.

CTL of B6 (H-2K^b) mice and B6·C-H-2^{bm_1} (H-2K^{bm_1}) and B6-H2-^{bm_3} (H-2K^{bm_3}) mutants can react against antigens, one with another, in the cytotoxic effect of immune lymphocytes (CEL) test. B6.C-H-2^{bm_1}, immune against B6, kill not only B6 targets, but also B6-H-2^{bm_3} [8]. After adsorption of B6.C-H-2^{bm_1} effector cells, immune against B6, on a monolayer of B6-h-2^{bm_3} cells activity against the latter is lost, whereas that against B6 is preserved [10]. On the basis of these **results Gieb et al.** [10] postulate the existence of more than one clone of CTL responsible for the reaction *in vitro* against H-2K antigen in the CEL.

Activity of CTL *in vivo* against H-2K antigen was studied in the present investigation by the use of mice tolerant to products of the H-2K^b gene and its mutant alleles.

EXPERIMENTAL METHOD

C57BL/6(B6) (H-2K^b) mice and mice of mutant lines derived from B6 as a result of mutations of the H-2K^b gene — Hz1, or B6.C-H-2^{bm_1} (H-2K^{bm_1}), M505, or B6-N-2^{bm_3} (H-2K^{bm_3}) and H-2^{bf}, or B6.C-H-2^{bm_4} (H-2K^{bm_4}) wereused. Mice of B6.C-H-2^{bm_1} and B6.C-H-2^{bm_4} lines were obtained from Bailey, B6-H-2^{bm_3} from Blandova. Mutant lines and their haplotypes in the investigation are described in accordance with the new nomenclature suggested at the First International Working Conference on H-2 mutations [11].

Tolerance to alloantigens was induced in newborn mice (not over 24 h) by intraperitoneal injection of $50 \cdot 10^6$ spleen cells. The donors of the cells were adult F_1 hybrids obtained by crossing mice of the recipient line and of another line, to the antigens of which tolerance had to be induced. The animals were considered to be tolerant if a skin graft of a mouse of the tolerogenic line was not rejected and did not lose its hair for more than 100 days [10]. The skin was grafted by the usual method [6] in Medvedev's modification [4].

EXPERIMENTAL RESULTS

Three groups of mice were studied: B6.C-H-2^{bm_1} (m-B6) (i.e., B6.C-H-2^{bm_1}, tolerant to B6), B6-H-2^{bm_3} (m-B6.C-H-2^{bm_1}). Skin from mice of the test lines was grafted onto these animals. The results of these experiments are given in Table 1.

As Table 1 shows, induction of tolerance to H-2K^b antigen in B6.C-H-2^{bm_1} mice significantly reduced the reactivity of this mutant against H-2K^{bm_3} antigen. B6.H-2^{bm_3} mutants, tolerant to H-2K^{bm_1}, rejected skin from B6(H-2K^b) and B6.C-H-2^{bm_4} (H-2K^{bm_4}) mice much later

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TABLE 1. Rejection of Skin Allografts by Tolerant Recipients

Group	H-2K recipient	H-2K tolerogen	H-2K skin donor	Time of rejection by tolerant recipients, days	Times of rejection by intact recipients, days
1	bm 1	b	b bm 3	>100 23, 24, 28, 30, 31, 35, 40	14,6 (2) 14,0 (2)
2	bm 3	bm 1	bm 1 b bm 4	>100 28, 29, 30, 42 22, 26, 29, 31	15,0 (2) 19,0 (2) 17, 18, 18, 19
3	b	bm 3	bm 3 bm 1	>100 13, 13, 14, 15	13,3 (2) 12,7 (2)

Legend. Full names of lines of mice given in text (number of mice in parentheses.)

than intact animals. Conversely, induction of tolerance to H-2K^{bm_3} antigen in mice of the original B6 line did not affect their activity against H-2K^{bm_1} antigen.

The tolerant animals studied, it will be recalled, were chimeras, whose immune system includes, not only its own cells, but lymphoid cells of hybrids by means of which tolerance was induced. However, their presence does not interfere with the considerable decrease in reactivity of the mice of the first two groups (Table 1, graphs 1 and 2). There was no change in reactivity in group 3.

The results can be interpreted as follows. The molecule of the H-2K^b antigen carries several determinants, recognizing CTL *in vivo*. B6.C-H-2^{bm_1}, B6-H-2^{bm_3}, B6.C-H-2^{bm_4} mutants (all mutations of the gain—loss type [11]) each have one (or more) modified determinants. These mutants are not identical [11] and, consequently, different CTL determinants are changed in them. Because of this, each mutant has at least two determinants that distinguish it from the other: One modified as a result of mutation and one not modified, but which is modified in the second mutant. During rejection of a skin graft of one mutant by the other, a separate CTL population reacts against each determinant. The CTL population acting against the unchanged determinant is eliminated during the induction of tolerance in the mutant to B6 or to the antigen of the other mutant. This accounts for the decrease in reactivity of tolerant mutants of groups 1 and 2 (Table 1). Induction of tolerance in mice of the original B6 line to one of the mutants inactivates the population of immunocompetent cells directed against the modified determinant only. On rejection of the graft of the other mutant, the participation of CTL of this population is unnecessary, for the graft is rejected by the tolerant animals in the same way as by the intact recipient (Table 1, group 3).

Partial complementation of allelic genes H-2K^{bm_1} and H-2K^{bm_3} during rejection of a B6 graft by (B6.C-H-2^{bm_1} × B6-H-2^{bm_3})F$_1$ hybrids [2] is perhaps indirect evidence of polyclonal heterogeneity of the CTL *in vivo*. During induction of tolerance to alloantigens in newborn animals, elimination of the corresponding clones of immunocompetent cells predominates [5], so that the formation of tolerance can be regarded as the *in vivo* analog, although not of course absolute, of immunoadsorption of CTL on a cell monolayer *in vitro*. In this light the present results agree with those of analysis of CTL activity *in vitro* using adsorption of effectors on a monolayer of macrophages [1, 3, 8, 10]. These results can be explained in two ways: Either the product of the H-2K gene contains several CTL-determinants, against each of which a separate clone of CTL reacts *in vitro* [1, 3, 9, 11], or the CTL receptors are distinguished not by specificity, but by affinity for the antigen. This last explanation is in harmony with the view that there is one CTL determinant [1, 3].

The writers are inclined to explain these results on the basis of clonal heterogeneity of cytotoxic T lymphocytes in the reaction *in vivo*. However, a different interpretation of these results cannot be completely ruled out.

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