Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 617–620, January 1989
Immunology

Recognition of xeno-(HLA, SLA) major histocompatibility complex antigens by mouse cytotoxic T cells is not H-2 restricted: A study with transgenic mice

(T-cell receptor)

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Communicated by Donald C. Shreffler, September 29, 1988

ABSTRACT **Cytotoxic T lymphocytes (CTLs) recognize antigens in the context of major histocompatibility complex (MHC) class I gene products. The T-cell receptor (TCR) that mediates this MHC-restricted antigen recognition recognizes short peptide fragments rather than the intact antigen. Presentation of peptides to the TCR may thus be a major function of the MHC. An intriguing question emerging from this model is whether peptide presentation also applies to foreign MHC antigens and which of the available MHC molecules can present preferentially the peptides of the foreign MHC molecule. Allo- and xenoreactive CTLs might either recognize native MHC class I molecules or peptides presented by self MHC or by the foreign class I MHC itself. The finding that synthetic peptides corresponding to MHC class I regions are recognized by allo- and xenoreactive CTLs suggests that recognition of foreign MHC by CTLs might involve degraded fragments presented by syngeneic class I molecules. We used MHC transgenic mice as a tool to study these questions. The CTL responses against human (HLA) antigen B27 were analyzed by using HLA-B27 transgenic mice with various *H-2* haplotypes. We report here that mouse xeno-MHC-specific (anti-B27) CTLs are perfectly able to kill human and mouse cells expressing the appropriate xenoantigen and that in primary and secondary responses to xeno-MHC, the mouse T-cell repertoire does not use self-H-2 as a restriction element. Absence of H-2 restriction was confirmed by the lack ($<1/10^6$) of H-2-restricted HLA-specific CTL precursors. Therefore, H-2-restricted recognition of xeno-MHC antigens cannot be generalized as part of a classical MHC class I-specific response. These results indicate that xenoreactive CTLs usually recognize intact MHC molecules or MHC peptides preferentially presented by their native MHC molecule. We suggest the latter possibility.**

In recent years, it has been established that peptides derived from processed antigens are bound to the peptide-binding site of major histocompatibility complex (MHC) molecules and are then presented to the T-cell receptor (TCR). The crystallographic structure of a class I molecule shows the peptide-binding site as a deep groove between the α-helices of the α_1 and α_2 domains (1, 2). The observation that the majority of MHC polymorphic residues are located in the peptide-binding site suggests that alloreactivity may reflect the recognition of a complex between the allo-MHC and some peptide (2). Such a peptide could be derived from the foreign MHC itself or from another molecule. Alternatively, self MHC molecules expressed on the membrane may bind the foreign MHC peptides. A consequence of the latter possibility would be that the recognition of the foreign MHC peptide is restricted by self MHC. However, the possibility remains that the TCR recognizes native allo- or xeno-MHC molecules rather than peptides.

In the mouse, syngeneic H-2-restricted recognition of HLA antigens has been frequently reported (3–7). Most of the experiments were done with selected cytotoxic T-lymphocyte (CTL) clones and lines raised against HLA-transfected mouse cells. Such CTLs might represent only a minimal part of the T-cell repertoire. Therefore, we examined whether mouse bulk CTL populations, directed against xeno-MHC HLA molecules, were restricted by the mouse MHC.

MATERIALS AND METHODS

Mice. The mouse strains were maintained at our institute. The origin and functional and biochemical analysis of the HLA-B27 and SLA-PD1 (human and porcine) xeno-MHC products in transgenic mice have been described (8–10). $H\text{-}2^b$, $H\text{-}2^q$, $H\text{-}2^k$, and $H\text{-}2^d$ B27dTGM (double transgenic mice with human β_2-microglobulin and HLA-B27K genes) were obtained from crosses between B27dTGM mice and B10, B10.Q, B10.BR, and B10.D2(n) mice, respectively. The SLA-PD1sTGM (single transgenic mice with the PD1 allele of SLA) are of B10 ($H\text{-}2^b$) origin (8). $H\text{-}2^k$ SLA-PD1 TGM were obtained from crosses to B10.BR ($H\text{-}2^k$) mice.

CTLs. Spleen cells from naive B10, B10.Q, B10.BR, or B10.D2 mice were cocultured with the same number of irradiated (2300 rad; 1 rad = 0.01 Gy) spleen cells from H-2-matched B27dTGM or PD1sTGM. This was done in 50 ml of Iscove's modified Dulbecco's medium, supplemented with 10% (vol/vol) fetal calf serum and penicillin (100 units/ml), streptomycin (100 μg/ml), and 5×10^{-5} M 2-mercaptoethanol. Autologous transgenic lymph node cells were cultured in the same medium and used as target cells. After 5 days, the responding CTLs were collected and tested in the ^{51}Cr release assay on human transgenic and nontransgenic cells stimulated for 3 days with concanavalin A (2.5 μg/ml). Responding CTLs were incubated with ^{51}Cr-labeled target cells at the effector/target ratio indicated.

For antibody-blocking experiments, target cells were incubated for 30 min on ice with 100 μl of monoclonal antibody (mAb) (1:10) and washed three times with medium before incubation with CTLs. The anti-HLA mAb W6/32 and anti-H-2 mAb Q-1 (11) were used, because they can effectively inhibit allo-CTLs and do not crossreact with $H\text{-}2^k$ and HLA-B27, respectively. Lysis of target cells expressing SLA-PD1 was blocked by PD1-specific mAb 74-11-10 (12). In cold target inhibition studies, 1×10^6 human cells, with or without HLA-B27, were incubated with mouse CTLs for 1 hr. The percentage of specific ^{51}Cr release was calculated as

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Abbreviations: mAb, monoclonal antibody; CTL, cytotoxic T lymphocyte; MHC, major histocompatibility complex; TCR, T-cell receptor; CTLpf, CTL precursor frequency.

(release in the presence of CTL spontaneous release)/(release in 10% Triton X-100 spontaneous release) × 100%. The spontaneous release in medium from labeled target cells ranged from 20% to 30% of the maximal release produced by Triton X-100.

For the primed CTL response, responder mice were injected intraperitoneally with one injection of 5×10^6 lymph node cells 3 weeks before the *in vitro* assay. CTL precursor frequencies (CTLpf) were estimated in limiting-dilution analysis by techniques described elsewhere (13).

Human Lymphocytes. HLA-typed lymphocytes were isolated from human peripheral blood by flotation on Ficoll-Isopaque and stored in liquid nitrogen until used. HLA types of the human cells were HLA-A2, -8; B27K, -27K; C2, –; DR2, -5 (27, 27): HLA-A3, -21; B27K, -61; C2, -2; DR8, -5 (27, –): HLA-2, -31; B18, -62; C3, –; DR4, -8 (–, –): HLA-A2, -28; B5, -12; C4, – (–, –).

RESULTS

The CTL response against xenogeneic MHC determinants on cells of mice double transgenic for the human genes HLA-B27K and β_2-microglobulin (B27dTGM) was studied. HLA-B27-specific CTLs were elicited by stimulation *in vitro* of $H\text{-}2^b$, $H\text{-}2^q$, $H\text{-}2^k$, or $H\text{-}2^d$ spleen cells (Fig. 1) with irradiated cells from B27dTGM cells of matched *H-2* haplotypes. The specificity of the CTL population(s) was tested on HLA-B27-positive and -negative human cells (Fig. 1 A, C, and D). The anti-HLA-B27 CTLs were able to kill only HLA-B27-positive human cells and the level of specific lysis was dependent on whether the target cells were homozygous or heterozygous for HLA-B27 (Fig. 1*A*). These results already show that the recognition of HLA-B27 xenogeneic MHC molecules by mouse CTLs occurred in a non-H-2-restricted way. The same CTL population(s) was perfectly able to kill HLA-B27-positive H-2-matched cells, whereas HLA-B27-negative H-2-matched cells were not lysed. Moreover, the same CTL populations were examined on H-2-mismatched B27dTGM mouse cells (in each experiment on one or two H-2-mismatched B27dTGM target cells; Fig. 1 *A, B, C,* and *D*, respectively). Again, in all combinations, cells expressing HLA-B27 were lysed, whereas no lysis was observed on HLA-B27-negative cells. No H-2 restriction in the recognition of HLA-B27 was found.

In the combination $H\text{-}2^k$–anti-$H\text{-}2^k$B27dTGM, the specificity of the CTL recognition was analyzed by antibody blocking and cold target inhibition experiments. Fig. 2*A* shows that lysis of B27dTGM cells could be blocked by anti-HLA but not by anti-H-2 antibodies and, second, that human B27-positive cells could block lysis of B27dTGM mouse cells. Fig. 2*B* shows the same but for the lysis of B27dTGM cells with the $H\text{-}2^q$ haplotype. Hence, recognition of B27 on H-2-matched ($H\text{-}2^k$) and H-2-mismatched ($H\text{-}2^q$) cells could be completely inhibited by anti-HLA antibody and by B27-positive human cells.

Furthermore, we estimated in limiting-dilution analysis the CTLpf for H-2-restricted and nonrestricted response to B27dTGM cells. The CTLpf in $H\text{-}2^b$ and $H\text{-}2^q$ mice specific for H-2-matched and -mismatched B27dTGM cells is ≈1 in 10^5 spleen cells. This value is ≈100 times lower than the CTLpf seen in naive animals after allogeneic stimulation. The CTLpf specific for H-2-matched transgenic cells (i.e., self H-2-restricted) in the same $H\text{-}2^b$ and $H\text{-}2^q$ mice was undetectable ($<1/10^6$). Taken together, these results show that mice are perfectly able to generate xeno-MHC-specific CTLs in primary response against transgenic cells.

Analogous experiments were performed also in primed mice. Fig. 3*A* shows that $H\text{-}2^k$–anti-$H\text{-}2^k$B27dTGM CTLs from mice primed by one injection of B27dTGM cells lysed specifically B27-positive human cells and $H\text{-}2^k$B27dTGM cells. Moreover, H-2-mismatched $H\text{-}2^q$B27dTGM and $H\text{-}2^b$B27dTGM cells were also lysed. Compared with the results from unprimed mice (Fig. 1), the percentage specific lysis was about 2 times higher with CTLs from *in vivo* primed mice. Still, these bulk CTLs could also be blocked by anti-HLA and human B27-positive cells (Fig. 3*B*).

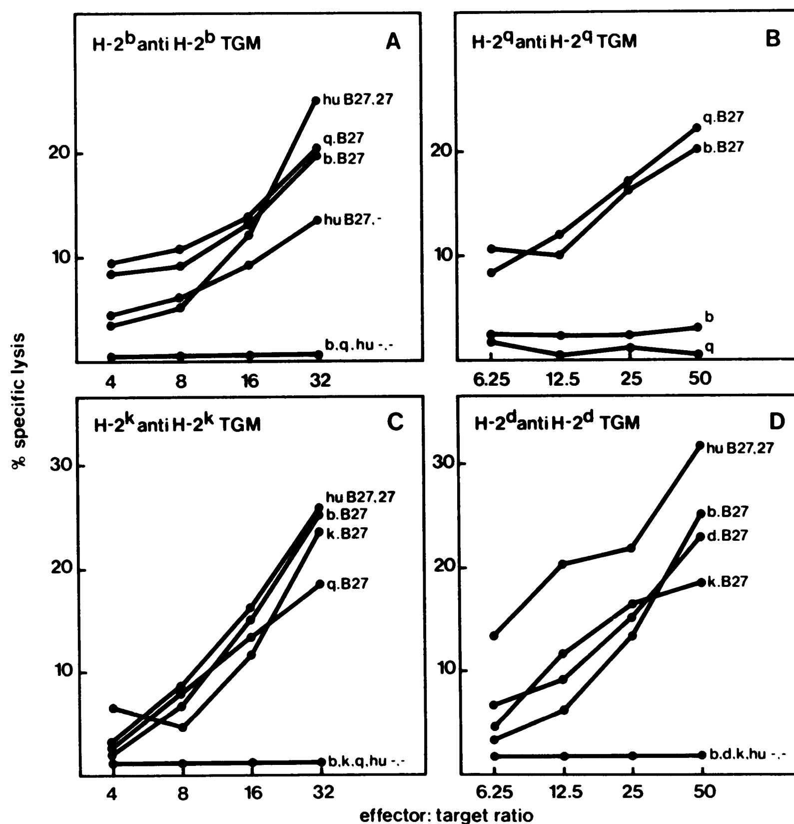

FIG. 1. Recognition of HLA-B27 on B27dTGM mouse cells and on human cells with HLA-B27. CTLs were obtained by stimulation with H-2-matched B27dTGM cells in $H\text{-}2^b$ (*A*), $H\text{-}2^q$ (*B*), $H\text{-}2^k$ (*C*), and $H\text{-}2^d$ (*D*) responders. The target mouse cells are designated b, q, k, d without HLA-B27 and b.27, q.B27, k.27, and d.B27 with HLA-B27. Human cells are designated hu B27,27; hu B27,–; and hu –,–; as homozygous, heterozygous, or negative for HLA-B27. The complete HLA phenotype of human cells is given in *Materials and Methods*.

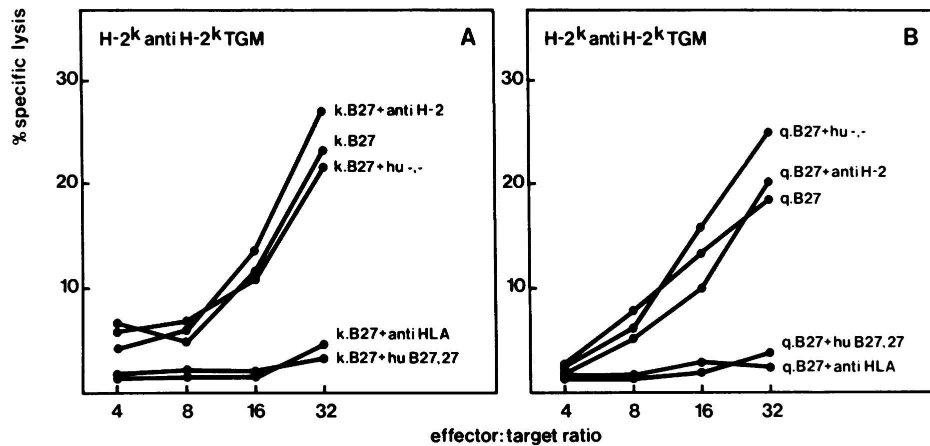

FIG. 2. Recognition of HLA-B27 on B27dTGM cells and inhibition of lysis by anti-HLA antibodies (k.B27 + anti-HLA) and human cells with HLA-B27 (k.B27 + hu B27,27). (*A*) The target cells are matched with the H-2 type of stimulator and responder ($H\text{-}2^k$). (*B*) The target cells are H-2-mismatched ($H\text{-}2^q$). The designation of target cells is the same as for Fig. 1.

H-2-nonrestricted recognition of porcine MHC (SLA) class I antigen (PD1) on transfected mouse cells was also reported (8). We performed experiments, analogous to those reported above for B27dTGM cells, also with SLA(PD1)sTGM cells. $H\text{-}2^b$–anti-$H\text{-}2^b$PD1sTGM CTLs lysed $H\text{-}2^b$- and $H\text{-}2^k$PD1sTGM cells. Similarly, $H\text{-}2^k$–anti-$H\text{-}2^k$PD1sTGM CTLs lysed $H\text{-}2^k$- and $H\text{-}2^b$PD1sTGM cells (data not shown). In both combinations, anti-SLA antibodies could block the lysis of the PD1sTGM cells.

Taken together, these results show that in naive as well as in primed mice recognition of xenogeneic MHC antigens by mouse CTLs appeared not to be restricted by self H-2 and occurred equally well on cells of various *H-2* haplotypes. Hence, while recognition of foreign (viral) and self non-MHC peptides appears regularly restricted by self MHC antigens, this rule does not apply to recognition of xeno-MHC peptides.

DISCUSSION

Recognition of foreign antigens, like haptens, viral, tumor, or minor histocompatibility antigens by T cells, is restricted by self MHC. The T-cell precursor frequency is low and priming *in vivo* is necessary before stimulation *in vitro*. This is in contrast with the nonrestricted recognition of allogeneic MHC determinants. The T-cell precursor frequency is high and stimulation occurs directly in primary lymphocyte cultures. It has been hypothesized that when foreign MHC antigens are seen as alloantigens by T cells, they are almost invariably associated with self or foreign non-MHC peptides (14). This interpretation argues that when T cells are confronted with allogeneic MHC determinants, they will respond to the myriad of complexes formed by non-MHC surface structures and the allogeneic MHC. This might explain the high frequency of such alloreactive T cells (15–17).

Some experiments that showed the activation of T cells by purified MHC antigens attached to synthetic beads suggest that alloreactive T cells recognize MHC alone (18). However, the possibility that these T cells recognize a peptide adduct persistently associated with purified soluble HLA antigens (1, 2) has not been ruled out. The observation that HLA-A2-specific CTLs were able to recognize HLA-A2-derived peptides only when the peptides were presented by HLA molecules closely related to HLA-A2 (19) indicate that recognition of allogeneic MHC may include recognition of peptides preferentially presented by their native MHC molecule. The high frequency of alloreactive T cells must then be explained in terms of concentration of MHC epitopes on cell surfaces (20).

A number of previous reports have dealt with the recognition of HLA antigens by mouse CTLs (3–7). These authors have concluded that HLA is generally recognized by mouse T cells as a nominal antigen in the context of a self H-2 class I restriction element. This implies that xenogeneic MHC determinants are not recognized in the same way as alloantigens. This conclusion has been based on the reactivity pattern of some selected CTL clones and CTL lines obtained after priming *in vivo* with HLA-transfected cells. Xenogeneic responses induced in such a way are often accompanied by responses against viral, tumor, or "minor" antigens and are therefore not representative for a classical MHC class I-specific response.

In the experiments described here, the availability of HLA-B27 and SLA-PD1 transgenic mice allowed us to test whether xenoreactive CTLs, in analogy with alloreactive CTLs, preferentially recognize their antigens in a non-H-2-restricted way. Our results show that this is indeed the case. Although the xeno-CTLpf is much lower than the allo-CTLpf, the results show that xeno-MHC antigens are

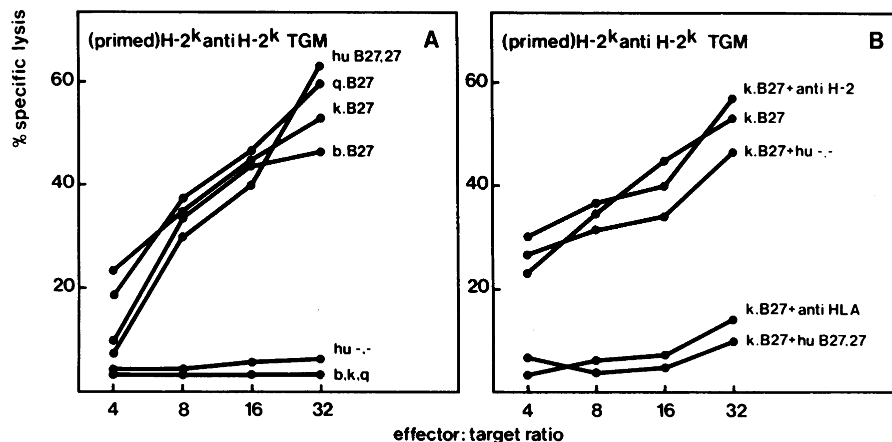

FIG. 3. (*A*) Recognition of HLA-B27 on B27dTGM mouse cells and on human cells with HLA-B27 by CTLs from primed responder mice. (*B*) Inhibition of lysis by anti-HLA antibodies and human cells with HLA-B27. The designation of target cells and inhibition experiments is the same as for Figs. 1 and 2.

recognized in bulk CTL cultures as alloantigens. It may well be that mouse CTLs recognize xenogeneic MHC as nominal antigens in the context of public restriction elements shared between various *H-2* haplotypes (H-2 public determinants). This possibility seems unlikely since (*i*) the induction of xenoreactive mouse CTLs occurs without priming, (*ii*) the same CTL cultures recognized HLA antigens on human cells, and (*iii*) recognition of viral antigens by bulk CTLs is normally restricted by private H-2 determinants. Furthermore, the finding that anti-B27dTGM CTLs are blocked by anti-HLA mAb that does not cross-react with H-2 antigens also suggests that recognition of xeno-MHC antigens is neither restricted by private nor by public H-2 determinants. Assuming that CTLs recognize peptides presented by MHC molecules (1, 2), we favor the view that MHC peptides are preferentially presented by their native MHC molecules. This does not rule out that some xenoreactive CTLs are responding to foreign MHC presented by self-MHC molecules. However, the frequency of such CTLs will be extremely low and that makes them the exception rather than the rule.

Note Added in Proof. According to the new HLA nomenclature B27K is a previous equivalent for B27.2 (see ref. 21).

We thank Dr. David Sachs for providing us with SLA-PD1 TGM breeding couples and hybridoma cells producing mAb 74-11-10. Breeding pairs of B27 transgenic mice were generously provided by Dr. H. L. Ploegh and A. Berns. We thank Mrs. Jetty Gerritsen for typing the manuscript. This work was supported by the Foundation for Medical Research (MEDIGON), which is subsidized by the Netherlands Organization for the Advancement of Pure Research (Grants 509-087 and 509-110).

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