A Subpopulation of Mouse Cytotoxic T Lymphocytes Recognizes Allogeneic H-2 Class I Antigens in the Context of Other H-2 Class I Molecules

By Femina Kievits and Pavol Ivanyi

From the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and the Laboratory for Experimental and Clinical Immunology, University of Amsterdam, 1066 AK Amsterdam, The Netherlands

Summary

Recently, independent lines of evidence strongly suggested that peptides derived from one foreign major histocompatibility complex (MHC) molecule bound to another MHC molecule can give rise to multiple composite MHC complexes that are able to stimulate allo-(xeno)-reactive T cells. In this study, we describe that in vivo immunization of mice with cells mismatched with the recipient for a single class I antigen results in the induction of CD8+ cytotoxic T lymphocytes (CTL) specific for allogeneic class I locus products (Dd, Kd, Dq) in the context of other class I molecules (Kk, Dd, Kk) present on stimulator cells. Evidently, the target antigen for these class I-restricted alloreactive CTL is not the native class I molecule but peptides derived from endogenous processing of allogeneic class I products presented by class I molecules. Using a combination of limiting dilution and split-well analyses, we estimated for Kk-restricted Dq-specific alloreactive CTL a precursor frequency (CTLpf) that was ~10 times lower than the CTLpf for "classical" nonrestricted Dq-specific alloreactive CTL. These data suggest that H-2 class I peptides presented by intact H-2 class I molecules are allostimulatory, supporting the concept that the capacity for presentation of MHC peptides by MHC molecules constitutes a part of the allogeneic immune response.

Virus-specific T cells recognize peptides derived from viral proteins produced in the infected cell. These peptides are presented by self-MHC class I or II molecules, as indicated by functional (1, 2) and crystallographic data (3, 4). Consequently, T cells recognize nominal antigens only on cells expressing MHC molecules identical to those of the responder. This is in contrast to the apparently nonrestricted recognition of alloantigens as alloreactive T cells recognize MHC antigens on whichever cell.

Based on the information that T cell recognition of nominal antigens involves trimolecular interactions between receptors on T cells, antigenic peptides, and class I or II MHC molecules, several current models have been suggested to account for allorecognition (reviewed in references 5–7). From most of the models, it appears that MHC alloreactivity probably involves a tripartite structure consisting of an allogeneic MHC molecule, an endogenous peptide, and a TCR. However, this does not rule out that some alloreactive CTL may recognize MHC molecules in the absence of peptide adducts, i.e., recognizing "naked" class I MHC molecules (8). Recent studies on alloreactivity also suggest that alloreactive responses include the recognition of MHC peptides derived from one MHC molecule and being presented to T cells by a second MHC molecule (9–15). Additional examples of the presentation of MHC peptides by MHC molecules emerge from studies on the xenogeneic response of murine T cells to human MHC products (16–19). In some of these studies, CTL clones were isolated for which the recognition of xenogeneic MHC peptides was restricted by self-MHC, like the recognition of nominal antigens (17). In other studies, the preferential binding of xeno-MHC peptides by their native MHC molecules has been suggested (20).

Thus, at least a part of the MHC molecules on stimulator and target cells might be occupied by derivatives of other self-MHC molecules. An intriguing question emerging from this concept is whether such composite MHC molecules play a role in alloimmune response in vivo by the induction of CTL specific for peptides derived from MHC alloantigens and presented by other MHC molecules expressed either on responder or stimulator cells. We report here that a subset of CTL indeed recognized allogeneic class I MHC locus products in the context of other class I molecules. This population of class I-restricted alloreactive CTL was detected only after in vivo priming at a frequency that was ~10 times lower than the classical nonrestricted alloreactive CTL population. These experiments suggest a further example of the capacity...
of MHC molecules to present MHC peptides, and show that allorecognition is a complex phenomenon in which multiple T cell populations seem to play a role. The results might have consequences for allogeneic transplantations in sensitized recipients.

Materials and Methods

Mice. All mice were obtained from the Animal House of our institute and bred under specific pathogen-free conditions. Mice were primed by one intraperitoneal injection of 10^7 allogeneic spleen cells in PBS 3–6 wk before use.

CTL Analysis. Limiting dilution (LD) and split-well assays have been described in detail elsewhere (21). Briefly, for split-well analysis, 2,500 responder lymph node cells of naive or primed mice were cocultured with 50,000 irradiated (25 Gy) allogeneic spleen cells per well in the presence of 20 U/ml rat IL-2. For each split-well experiment, 96 wells were seeded. After 7 d, microcultures were divided into three (60-μl) or four (45-μl) aliquots and transferred into wells containing 140 or 155 μl of medium and 5,000 ^51Cr-labeled target cells. Culture medium was IMDM supplemented with 10% (wt/vol) FCS and 100 IU/ml penicillin, 100 μg/ml streptomycin, and 5 × 10^{-3} M 2-ME. After 4 h of incubation at 37°C, the supernatants were automatically harvested by Titertek systems and counted in a gamma counter. Responding cultures were defined as those in which the ^51Cr release values exceeded in the mean spontaneous ^51Cr release plus three times the SD. LD cultures were set up starting from 64,000 to 500 responder lymph node cells/well in 24 replicates. Stimulation, culture, and ^51Cr release conditions, as well as the splitting of LD cultures into four aliquots, were the same as for split-well analysis.

Target Cells. Spleen cells cultured for 3 d in the presence of LPS (30 μg/ml) were used as target cells. Before labeling, target cells were centrifuged on Ficoll. For blocking studies with H-2-reactive antibodies, target cells were incubated with 100 μl of hbrinoma culture supernatant for 30 min at 37°C followed by the same period on ice. Cells were washed twice with culture medium before the addition of CTL. For blocking studies with anti-CD8, target cells and CTL were incubated in the presence of 20 μl of anti-CD8 antibodies (1:1,000). Antibodies used in these studies were: anti-D^k (28.14.85 [22]), anti-K^k (36.7.5S [23]), and anti-Ly2.2-specific mAb (New England Nuclear, Boston, MA).

Calculation of CTL Precursor Frequencies (CTLpf). CTLpf were calculated by scoring wells with responding and nonresponding cultures. Minimal estimates of CTLpf were calculated using the jackknife version of the maximum likelihood procedure with a 95% confidence interval determined according to the method described by Strijbosch et al. (24). The correlation coefficient of the experiments given was >0.90. Computer programs for the above-described CTLpf calculations are available from the Computer Applications Group (Tilburg University, Tilburg, The Netherlands).

Results and Discussion

To analyze whether a fraction of alloreactive CTL recognize MHC alloantigens in the context of other MHC antigens, we compared primary and secondary alloreactive CTL responses of mice induced by a single H-2 class I difference.

Because CTL bulk cultures are in general not restricted by MHC, we generated CTL cultures at the level of precursor frequency. For this propose, lymph node cells from naive and primed mice were stimulated in microcultures with allogeneic spleen cells. Each microculture was split into three or four aliquots and tested for cytotoxicity against selected target cells.

Table 1 shows the split-well analysis of three independent experiments. Before priming, all CTL microcultures were reactive with target cells expressing the stimulating class I H-2 antigen. Target cell lysis was not dependent on non-MHC genes, i.e., background differences (Exp. 1) or coexpression of other class I H-2 molecules on responder, stimulator, or target cells (Exps. 1–3). These observations are general and reported for a broad field of classical alloreactive CTL research.

After priming, the majority of responding T cells exhibited the same reactivity pattern. However, another population of CTL lysed exclusively those target cells expressing in addition to the stimulating class I molecules, class I molecules shared by both responder and stimulator cells. It appears that after priming in vivo and restimulation in vitro, for a small population of CTL, the recognition of the alloantigen is restricted by syngeneic class I H-2 molecules. Obviously, recognition of alloantigens is restricted here by H-2 private determinants. The percentage of H-2 class I-restricted alloreactive CTL seems to be low, but it may well be that part of the restricted CTL were overgrown by the classical non-MHC-restricted alloreactive CTL. For that reason, we made an effort to block target cell lysis by H-2-specific antibodies. To ensure the involvement of CD8^+ T cells, only those microcultures were taken into account that were blocked by anti-CD8 antibodies. Table 2 shows that 49 microcultures contained CD8^+ T cells reactive with the stimulator cells. Of these 49 cultures, 28 were blocked by anti-D^k antibodies, suggesting a direct recognition of allogeneic D^k epitopes. 11 CTL microcultures were not blocked by anti-D^k but by K^k-reactive antibodies. Here, the K^k molecule seems to be involved in recognizing allogeneic D^k antigens. The peptide model valid for nominal antigen recognition provides the best model for explaining the specificity of the latter alloreactive CTL. The recognized alloantigen seems to be formed by K^k molecules associated with a class I peptide derived from D^k molecules. The K^k molecules on the target cells (LPS blasts) apparently contained sufficient amounts of D^k peptides to be lysed by the CTL. Evidently, D^k molecules are processed in the target cells and presented to K^k molecules by the endogenous pathway of antigen presentation. Interactions on the target cell membrane between T cell receptors and K^k molecules associated with D^k peptides will be blocked by K^k-reactive mAbs rather than by anti-D^k antibodies. Because B10.BR and B10.AKM mice differ only in class I D^k or K^k gene products, our findings suggest that a low percentage of the syngeneic class I H-2 molecules is occupied by peptides of other class I H-2 molecules. This has recently also been shown in the human system for class I HLA molecules of which the processed peptides were bound by HLA class II expressed on the same cell surface (13, 15). Hence, endogenous processing and presentation of class I peptides normally occurs in vitro and in vivo. However, our results in the mouse.
model suggest that for class I molecules, peptide presentation is more efficient after sensitization in vivo. Probably, expression of the minimal number of MHC/peptide complexes required for T cell activation is stimulated by priming and differs from the number of complexes necessary for target cell lysis (25, 26).

Several other publications demonstrated that MHC class I peptides can be recognized when presented by allogeneic (9, 10) or xenogeneic (16, 17) MHC molecules or that such peptides might inhibit allo- (11, 27) or xenoreactivity (28). In this context, it is of interest to know at which frequency CTL recognize such composite MHC antigens. Using a combination of LD and split-well analysis, we estimated the frequency of CTL precursors in lymph node cell populations before and after sensitization to a single class I difference. Table 3 shows that the frequency of class I-restricted alloreactive CTL is ~10 times lower than of the nonrestricted classical alloreactive CTLp and that the majority of restricted CTL is contributed by previously primed cells. Thus, in vivo immunization with allogeneic class I molecules stimulates CTL specific for composite determinants consisting of allogeneic class I peptides presented by other class I molecules. Together with the information that CTL are also able to recognize class I peptides presented by class II molecules (13, 15), it can be speculated that both class I and class II MHC molecules present either class I- or class II-derived peptides. Recently, it has been postulated that such complexes might play a role in the determination of T cell repertoires (15) or in T cell suppressor networks (29). Possibly, peptides derived from one MHC gene product will bind to the intact products of the same gene (20) and may in this way participate in elimination or inactivation of T cells with self-MHC specificities.

Taken together, our results demonstrate that the recognition of allogeneic class I antigens is for a limited number of CD8⁺ T cells restricted by class I MHC molecules. The low CTLp suggests that these restricted alloreactive CTL are only a minor part of the total T cell population and are therefore possibly serendipitously generated on the basis of a lower affinity for class I determinants.
Table 2. Split-well Analysis of Secondary Allo-(D9)-reactive CTL
in the Presence of CD8- or H-2-specific mAbs

| Responder       | Stimulator      | H-2 | Split-well reaction pattern of CTL microcultures |
|-----------------|-----------------|-----|-----------------------------------------------|
| B10.BR          | B10.AKM         | k   |                                               |

Inhibition by:

1. - 0 0
2. Anti-CD8 + +
3. Anti-D9 + 0
4. Anti-Kk 0 +

96 microcultures of alloreactive CTL were seeded as indicated in the legend to Table 1. Lymph node cells of primed mice were used as responder cells. +, blocking of target cell lysis; 0, no blocking of target cell lysis.

* Expressed is the number out of 96 CTL microcultures with the indicated split-well pattern of target cell lysis. Targets were: stimulator cells (1), stimulator cells in the presence of anti-CD8 mAbs (2), and stimulator cells previously incubated with D9- (3) or Kk- (4) specific mAbs.

Table 3. Precursor Frequencies of Primary and Secondary Allo-(D9)-reactive CTL

| Exp. | Responder B10.BR | Stimulator B10.AKM | H-2K | H-2D | Frequency* | 95% CI† |
|------|------------------|--------------------|------|------|------------|--------|

1s (before priming)

- Targets: R
  - S 3,788
  - S + anti-D9 89,909
  - S + anti-Kk 4,409

2 (after priming)

- Targets: R
  - S 2,392
  - S + anti-D9 25,900
  - S + anti-Kk 3,185

3 (after priming)

- Targets: R
  - S 3,257
  - S + anti-D9 32,171
  - S + anti-Kk 3,744

Wells were tested on responder (R), stimulator (S), and stimulator cells previously incubated with H-2-specific mAbs. Experiments are representative and independent examples.

* Reciprocal CTL precursor frequency.
† 95% Confidence interval.
§ CTLpf were estimated in individual mice by a combination of LD and split-well analysis.
‖ CTLpf and 95% CI are calculated using extrapolation to responder cell numbers not used in the LD assay.
We thank Walter J. Boerenkamp for his technical assistance and Mrs. J. Gerritsen for typing the manuscript. This work was supported by the Dutch Foundation for the support of MS Research (grant 87-9). F. Kievts is supported by a fellowship of the Royal Netherlands Academy of Arts and Sciences.

Address correspondence to Femia Kievts, c/o Publication Secretariat, Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, P.O. Box 9406, 1006 AK Amsterdam, The Netherlands.

Received for publication 22 January 1991.

References

1. Townsend, A.R.M., F.M. Gotch, and J. Davey. 1985. Cytotoxic T cells recognize fragments of influenza nucleoprotein. Cell. 42:457.
2. Townsend, A.R.M., J. Rothbard, F.M. Gotch, G. Bahadur, D. Wraith, and A.J. McMichael. 1986. The epitopes on influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined by short peptides. Cell. 44:959.
3. Bjorkman, P.J., M. A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, and D.C. Wiley. 1987. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature (Lond.). 329:512.
4. Brown, J.H., T. Jardetzky, M.A. Saper, B. Samraoui, P.J. Bjorkman, and D.C. Wiley. 1988. A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. Nature (Lond.). 332:845.
5. Korulisky, P., and J.M. Claverie. 1989. MHC-antigen interaction: What does the T cell receptor see. Adv. Immunol. 45:107.
6. Eckels, D.D. 1990. Allogeneic presentation of endogenous peptide or direct recognition of MHC polymorphism? Tissue Antigens. 35:49.
7. Lechler, R.I., G. Lombardi, J.R. Batchelor, H. Reinsmoen, and P.H. Bach. 1990. The molecular basis of alloreactivity. Immunol. Today. 11:83.
8. Elliot, T.J., and H.N. Eisen. 1990. Cytotoxic T lymphocytes recognize a reconstructed class I histocompatibility antigen (HLA-A2) as an allogeneic target molecule. Proc. Natl. Acad. Sci. USA. 87:5312.
9. Clayberger, C., P. Parham, J. Rothbard, D.S. Ludwig, G.K. Schoolnik, and A.M. Krensky. 1987. HLA-A2 peptides can regulate cytolysis by human allogeneic T lymphocytes. Nature (Lond.). 330:763.
10. Song, E.S., C.A. Olson, M. McMillan, and R.S. Goodenow. 1988. Allospecific cytotoxic T lymphocytes recognize an H-2 peptide in the context of a murine MHC class I molecule. Proc. Natl. Acad. Sci. USA. 85:1927.
11. Heath, W.R., A. Vitiello, and L.A. Sherman. 1989. Mapping of epitopes recognized by alloreactive cytotoxic T lymphocytes using inhibition by MHC peptides. J. Immunol. 143:1441.
12. Schendel, D.J. 1990. On the peptide model of allore cognition: cytotoxic T lymphocytes recognize alloantigen encoded by two HLA-linked genes. Hum. Immunol. 27:229.
13. Essakat, S., J. Fabron, C. de Preval, and M. Thomsen. 1990. Corecognition of HLA-A1 and HLA-DRW3 by a human CD4+ alloreactive T lymphocyte clone. J. Exp. Med. 172:387.
14. De Koster, S.H., D.C. Anderson, and A. Termijtelen. 1989. T cells sensitized to synthetic HLA-DR3 peptides give evidence of continuous presentation of denaturated HLA-DR3 molecules by HLA-DP. J. Exp. Med. 169:1191.
15. Chen, B.P., A. Madrigal, and P. Parham. 1990. Cytotoxic T cell recognition of an endogenous class I HLA peptide presented by a class II HLA molecule. J. Exp. Med. 172:779.
16. Maryanski, J.L., P. Pala, G. Corradin, B.R. Jordan, and J. Cerottini. 1986. H-2-restricted cytolytic T cells specific for HLA can recognize a synthetic HLA peptide. Nature (Lond.). 324:578.
17. Maryanski, J.L., R.S. Accolia, and B. Jordan. 1986. H-2-restricted recognition of cloned HLA class I gene products expressed in mouse cells. J. Immunol. 136:4340.
18. Holterman, M.J., and V.H. Engelhard. 1986. HLA antigen expressed on murine cells are preferentially recognized by murine cytotoxic T cells in the context of the H-2 major histocompatibility complex. Proc. Natl. Acad. Sci. USA. 83:9699.
19. Achour, A., B. Begue, E. Gomard, P. Paul, B. Sayagh, A. Van Pel, and J.P. Levy. 1986. Specific lysis of murine cells expressing HLA molecules by allospecific human and murine H-2-restricted anti-HLA T killer lymphocytes. Eur. J. Immunol. 16:597.
20. Kievts, F., J. Wijffels, W. Lokhorst, and P. Ivanyi. 1989. Recognition of xenogen (HLA, SLA)-MHC antigens by mouse cytotoxic T cells is not H-2-restricted. A study with transgenic mice. Proc. Natl. Acad. Sci. USA. 86:617.
21. Kievts, F., W.J. Boerenkamp, W. Lokhorst, and P. Ivanyi. 1990. Specificity and frequency of primary anti-HLA cytotoxic T lymphocytes in normal and HLA-B27.2-, HLA-B27.5-, and HLA-Cw3-transgenic mice. J. Immunol. 144:4513.
22. Ozato, K., T.H. Hansen, and D.H. Sachs. 1980. Monoclonal antibodies to mouse MHC antigens. J. Immunol. 125:2473.
23. Ozato, K., N. Mayer, and D.H. Sachs. 1980. Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 Ia antigens. J. Immunol. 124:533.
24. Strijbosch, L.W.G., W.A. Buurman, R.J.M.M. Does, P.H. Zinken, and G. Groenewegen. 1987. Limiting-dilution assay: experimental design and statistical analysis. J. Immunol. 97:133.
25. Demotz, S., M. Howard, and A. Sette. 1990. The minimal number of class II MHC-antigen complexes needed for T cell activation. Science (Wash. DC). 249:1028.
26. Harding, C.V., and E.R. Unanue. 1990. Quantitation of presenting cell MHC class II/peptide complexes necessary for T cell stimulation. Nature (Lond.). 346:574.
27. Parham, P., C. Clayberger, S.L. Zorn, P.S. Ludwig, G.K. Schoolnik, and A.M. Krensky. 1987. Inhibition of alloreactive cytotoxic T lymphocytes by peptides from the α2 chain of HLA-A2. Nature (Lond.). 325:625.
28. Maryanski, J.L., P. Pala, J.C. Cerottini, and G. Corradin. 1988. Synthetic peptides as antigens and competitors in recognition by H-2-restricted cytolytic T cells specific for HLA. J. Exp. Med. 167:1391.
29. Janeway, C.A. 1989. Immunotherapy by peptides? Nature (Lond.). 341:482.