XENOGENEIC HUMAN ANTI-MOUSE T CELL RESPONSES ARE DUE TO THE ACTIVITY OF THE SAME FUNCTIONAL T CELL SUBSETS RESPONSIBLE FOR ALLOSPECIFIC AND MAJOR HISTOCOMPATIBILITY COMPLEX-RESTRICTED RESPONSES*

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The T cell recognizes and responds to non-major histocompatibility complex (MHC)[1] antigens only when presented in some association with the polymorphic allele specific determinants of self-MHC. The cytotoxic response is restricted to allele-specific determinants encoded by class I MHC loci (1) and the helper response is restricted to those encoded by class II (2).

A high frequency of T cells responds to allogeneic MHC. These responses against allogeneic MHC are largely directed against the same allele-specific determinants and show the same bias toward an association between killer function and class I MHC determinants and helper function and class II (3).

T cell subsets can also be defined by cell surface molecules, the Lyt antigens in the mouse (4), and the Leu antigens in the human (5). T cells that recognize class I antigens display Lyt-2 (6) in the mouse or Leu-2 in the human (7) and their function is blocked by these reagents (8–10). T cells that recognize class II determinants are Lyt-2 negative or low (6) or in the human are Leu-2a negative, Leu-3a positive (7, 11, 12).

The correlation between T cell function and MHC class takes the form of a bias and is not absolute. The correlation between surface antigen phenotype and MHC class recognized may be absolute (6, 12).

The similarities in the characteristics of the T cells that respond to self-MHC plus nominal antigen X and to allogeneic MHC are striking. In the allogeneic response one would expect T cells that would respond to the nonpolymorphic parts of MHC molecules to be missing because of self-tolerance. The restriction to the polymorphic determinants in the response to self plus X requires some other explanation. The association between cell surface molecules (revealed by anti-Lyt and anti-Leu reagents) and the class of MHC antigen presented in both the self plus X and the allogeneic response might be taken to suggest some features of a cell interaction that can take place equally well between syngeneic and allogeneic cells.

[1] *Abbreviations used in this paper:* Con A, concanavalin A; CTL, cytotoxic T lymphocytes; IL-2, interleukin 2; LPS, lipopolysaccharide; MHC, major histocompatibility complex; PBL, peripheral blood lymphocytes.

We have therefore examined these same features, specificity for common or polymorphic epitopes, associations of function with MHC subregion, and expression of Leu differentiation markers with function, in a xenogeneic response, that of human peripheral blood T cells responding against mouse T-depleted spleen cell populations. We could find no significant differences in the characteristics of the xenogeneic compared with the allogeneic response in any of the features examined.

On the basis of these results we propose that there are two components to T cell recognition of MHC antigen. One component is an interaction with an area on the molecule that does not carry the polymorphic determinants and which is conserved between species. This is the interaction that distinguishes between class I and class II MHC molecules. The other component is the recognition of the polymorphic determinants of the MHC molecule. It is important to note that these two components do not correspond to the two recognition events that figure in the classic one receptor/two receptor debate. In our model, the first component is a separate recognition step not previously considered and it is the second component, that which recognizes the polymorphic part of MHC, be it syngeneic, allogeneic, or xenogeneic, that may involve one or two receptors in the traditional sense. The model that we propose does not address this issue.

Materials and Methods

Mice. All strains of inbred mice were bred in our colony at the University of California at San Diego. Original breeding pairs were obtained from The Jackson Laboratory, Bar Harbor, ME, and from Dr. Donald Shreffler (Washington University School of Medicine, St. Louis, MO).

Human Peripheral Blood Lymphocytes (PBL). Responder cells were obtained from healthy volunteers and purified by Ficoll-Hypaque flotation (13).

Mouse Stimulator Cells. These were from pooled spleen cells. Spleen cells were treated with two monoclonal anti-Thy-1.2 reagents (F7D5 of P. Lake and HO-13.4 of Marshak-Rothstein). Resulting non-T cells were mitomycin treated and used as stimulators.

Xenogeneic Cultures. Primary cultures were set up with 10^7 human PBL plus 3–4 × 10^7 mouse stimulators in a total volume of 20 ml RPMI supplemented with 5% selected fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml); L-glutamine (2 mM), and 5 × 10^{-5} M 2-mercaptoethanol. Twice during the first 7–10 d of culture, 25% of the medium was replaced.

Cell-mediated Lympholysis. Primary cultures were harvested after 8–10 d and passed through nylon columns. Recovered T cells were cultured in Linbro wells (Linbro Chemical Co., Hamden, CT) in a total volume of 2 ml at 1 × 10^6/ml with mouse stimulators prepared as for primary culture at 3 × 10^6/ml. After 4 d cells were harvested and cytotoxicity was determined on mouse splenic ^{51}chromium-51-labeled (Cr^{51}) concanavalin A (Con A) (2 mg/ml) plus lipopolysaccharide (LPS) (10 μg/ml)-induced blast targets.

Production of Interleukin 2 (IL-2). Primary human anti-mouse cultures were harvested after 10 d and cells were passed through nylon wool (see section above). Effluent T cells were untreated or treated with mitomycin. Human T cells at 5 × 10^5/ml were mixed with mouse stimulators at 2–3 × 10^6/ml in 1-ml Linbro cultures. Culture supernatants were collected after 24 h and tested for IL-2 activity by their ability to support the growth of an IL-2-dependent NK line (14) and by their ability to co-stimulate the Con A-induced proliferation of murine thymocytes (14). Similar results were obtained in both assays.

Blocking with Anti-Leu-2a and Anti-Leu-3a Reagents. Monoclonal antibodies to Leu-2a and Leu-3a (5, 8) were obtained from Becton, Dickinson & Co., Oxnard, CA. These reagents were added directly to the assays of cytotoxic effects and to the cultures for the production of IL-2. These reagents were present at ≤5% final concentration corresponding to an antibody concentration of 2 μg/ml.

Results

Specificity of the Cytotoxicity Response. Human PBL were cultured for 7 d with mitomycin C-treated, T-depleted mouse spleen cells as described in Materials and Methods. The human cells were harvested, washed, and resuspended in fresh medium. They were restimulated with mouse stimulator cells that were syngeneic with the initial stimulators, syngeneic at K and D only, syngeneic at I only, or totally allogeneic. The cells were harvested after another 4 d and assayed on ^{51}Cr-labeled targets which were syngeneic with the stimulator cells used in the second culture period. This experimental protocol was thus designed to assay the specificity of responder T cells that were restimulatable in the second culture.

As can be seen from Fig. 1, a strong cytotoxic response was obtained when the secondary stimulator cells were syngeneic with the stimulators used in the initial culture. In the experiment shown, an equally good cytotoxic response was also seen when the secondary stimulators [B10.T(6R)] shared only the K and D alleles with the primary stimulators. In contrast, no measurable response was obtained with allogeneic
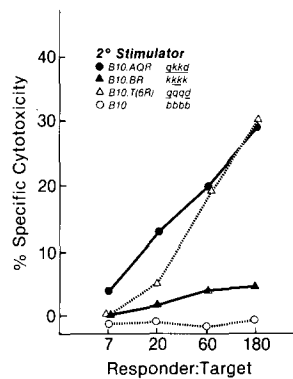

Fig. 1. Specificity of restimulation of human anti-mouse response. Human PBL were stimulated in vitro with mitomycin-treated non-T cells from the spleens of B10.AQR mice. After 10 d, cultures were harvested, nylon wool passed, and restimulated with non-T cells from B10.AQR (●); B10.BR (▲); B10.T(6R) (△); or B10. (○) mice. Cytotoxicity on homologous ^{51}Cr-labeled mouse targets was determined after 4 d of culture. The MHC haplotype of mouse strains at K.IA.IE/C.D are shown.
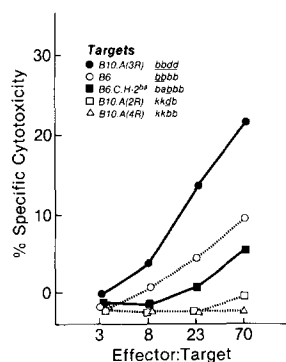

Fig. 2. Target specificity of secondary human anti-mouse CTL effectors. Human PBL were primed and restimulated in vitro with B10.A(3R) non-T cells. Cytotoxic effectors were measured on a panel of mouse targets: B10.A(3R) (●), B6 (○), B6.C.H-2^{ha} (■), B10.A(2R) (□), and B10.A(4R) (△).

(B10) cells as secondary stimulators, whereas only a small response was seen with B10.BR cells that share I region alleles. This basic pattern of results was seen in a series of experiments using PBL from five different human donors. In most cases it was an advantage to have the same I region as well as the same K/D in the restimulation (not shown). In some experiments stimulators differed at background (for instance, B10 vs. A backgrounds) and this did not affect the degree of cytotoxicity elicited (not shown).

The specificity of the cytotoxic T lymphocyte (CTL) effectors was investigated in a second experimental protocol in which human CTL were generated by stimulation and restimulation with the same mouse haplotype, B10.A(3R). These were assayed on a series of ^{51}Cr-labeled targets (Fig. 2). B10.A(3R) targets were killed efficiently, whereas allogeneic targets [B10.A(4R)] were not lysed. A trace amount of lysis was seen on a IE/C region-compatible target [B10.A(2R)]. B6, which shares K^b and IA^b, was lysed at an intermediate level. Targets from the B6.C.H-2^{ba} (bm-1) mutant were lysed slightly less than the wild-type B6. This latter result, seen in four out of five experiments, suggested that a portion of the human CTL raised against K^b [B10.A(3R)] killed K^b (B6) better than K^{ba} (B6.C.H-2^{ba}).

Specificity of the Induction of IL-2 Production. Although CTL effectors were specific for K or D alloantigens, human PBL were also able to respond to mouse I region antigens in an allele-specific manner and produce IL-2. In these experiments the production of IL-2 was measured during a 24-h culture period when human PBL were restimulated with mouse T cell-depleted spleen cells syngeneic or allogeneic to the original stimulator mouse cells. The human IL-2 was assayed by the proliferation of an IL-2-dependent mouse NK cell line.

In Fig. 3 it can be seen that significant IL-2 production was seen when B10.A(3R)-primed cells were restimulated with cells syngeneic at IA, that is cells from B10.A(3R), B6, and B6.C.H-2^{ba}. No IL-2 production was seen when cells used as stimulators were compatible only at D (B10.A) or were totally allogeneic [B10.A(4R)]. It is important to note that this result confirms the expectation that the IL-2 is produced by the human lymphocytes rather than the T-depleted mouse cells used as stimulators in the second culture. Similar results were seen in a series of experiments involving cells from four human donors and various combinations of mouse stimulators from H-2-recombinant strains, including B10.AQR-stimulated cells, which were successfully restim-

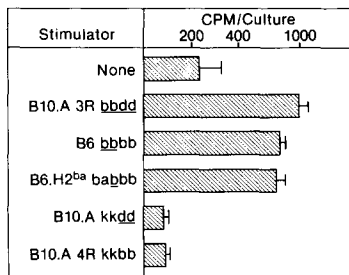| Stimulator	CPM/Culture 200 400 1000
None	
B10.A 3R bbdd	
B6 bbbb	
B6.H2ba babbb	
B10.A kkdd	
B10.A 4R kkbb	

FIG. 3. Specificity of restimulation of IL-2 production. Human PBL were primed in vitro with B10.A(3R). After 7 d, cultures were harvested, nylon wool passed, and restimulated with a panel of mouse non-T stimulators. Supernatants were harvested from triplicate cultures after 24 h and added at 15% final volume to an IL-2 assay. The background proliferation in the IL-2 assay without added factors was 189 cpm per culture.

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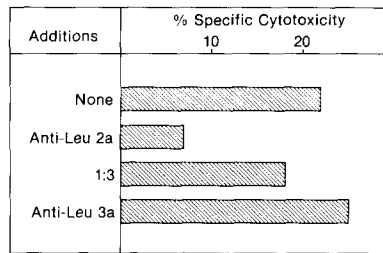| Additions	% Specific Cytotoxicity 10 20
None	
Anti-Leu 2a	
1:3	
Anti-Leu 3a	

Fig. 4. Anti-Leu-2a and anti-Leu-3a effects on cytotoxic killing. Human PBL were primed and restimulated in vitro with B10.A(3R) cells. Monoclonal anti-Leu-2a was added at a top concentration of 2.5% (1 μg/ml) and at a 1:3 dilution, and anti-Leu-3a was added at 2.5% (1 μg/ml) during the 4-hour assay. Targets were B10.A(3R) ^{51}Cr-labeled blast cells induced by Con A and LPS. A titration of cytotoxic effectors was tested for these conditions. Only the effect at an effector/target ratio of 50:1 is shown. The same pattern of blocking was seen at 17:1 and 6:1.

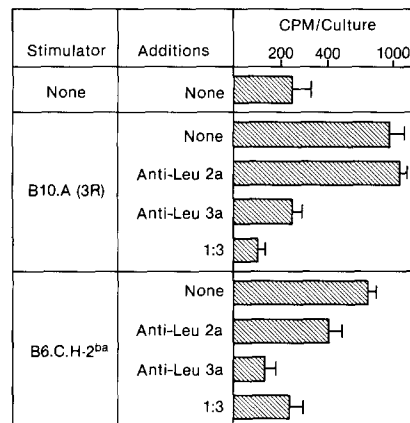| Stimulator	Additions	CPM/Culture 200 400 1000
None	None	
B10.A (3R)	None	
	Anti-Leu 2a	
	Anti-Leu 3a	
	1:3	
B6.C.H-2^{ba}	None	
	Anti-Leu 2a	
	Anti-Leu 3a	
	1:3	

Fig. 5. Anti-Leu-2a and anti-Leu-3a effects on IL-2 production. Human PBL were primed and restimulated in vitro with B10.A(3R) cells. Monoclonal anti-Leu-2a at 1% (0.4 μg/ml) and anti-Leu-3a at 1% (0.4 μg/ml) and 0.33% (1:3) were added to triplicate cultures during the generation of IL-2. IL-2 production was determined as in Fig. 4.

ulated with B10.AQR or B10.BR but not with B10.T(6R), and B10.AQR-stimulated cells, which produced IL-2 after restimulation with A.TL but not A.TH or B10.T(6R).

Specificity of Blocking with Anti-Leu Reagents. Anti-Leu reagents were tested for the blocking of the response of human lymphocytes to mouse MHC antigens.

The CTL Response. In this protocol anti-Leu-2a or Leu-3a reagents were present only during the CTL assay. It can be seen in Fig. 4 (which is representative of five similar experiments with different strain combinations) that CTL effectors raised by primary stimulation with B10.A(3R) restimulated with B10.A(3R) were blocked by the presence of anti-Leu-2a but not by anti-Leu-3a.

IL-2 Production. The same reagents had the opposite effect in blocking IL-2 production (Fig. 5). Thus the anti-Leu-2a that had markedly inhibited the CTL response to K and D antigens had no effect on the production of IL-2 to I region antigens. The anti-Leu-3a that had no effect on CTL activity (Fig. 4) now blocked IL-2 production in response to I region antigens. In these experiments human cells stimulated with B10.A(3R) in the first culture were restimulated with B10.A(3R) (upper panel) with B6.C.H-2^{ba} (lower panel). The anti-Leu reagents were present only

in the second culture period. Anti-Leu reagents added to the assay of IL-2 at the concentrations found in these supernatants had no effect on proliferation. Similar results were seen in a series of experiments using PBL from several donors.

Discussion

The results presented here show: (a) That human T cells respond strongly to the mouse MHC antigens. (b) That the response is predominantly to the allele-specific determinants. (c) That the bias in the association between T cell function and the class of MHC molecule that is recognized operates across species. CTL are generated to xenogeneic class I antigens and IL-2 producers to xenogeneic class II-encoded determinants. (d) That the cytotoxic response to xenogeneic class I MHC antigens is blocked by anti-Leu-2a and the response of IL-2-producing cells to xenogeneic class II antigen is blocked by anti-Leu-3a.

The first and second observations confirm the earlier studies of Lindahl and Bach (15, 16). There is little or no response to the non-MHC antigens of the mouse or to the nonpolymorphic determinants of the mouse MHC. This is in marked contrast to most MHC-specific antibodies raised across species. Many of these antibodies are broadly polymorphic or monomorphic with only a few being allele specific. Cross-reactions on species not closely related are rare (17).

The size of the in vitro T cell response suggests that the frequency of the T cells that recognize the mouse allele-specific determinants is closer to that of T cells that recognize allele-specific determinants within the species than to that of T cells that recognize non-MHC antigens.

The killing of mouse targets cells in the CTL assays strongly argues that the human CTL effectors are able to recognize and use the mouse MHC molecules instead of human MHC molecules because the possibility of representation of mouse MHC determinants in conjunction with human MHC is minimized under these conditions.

These observations, surprising in themselves, are made more remarkable by the finding that the T cell makes the same "functional" distinction between class I and class II antigen as is seen within the species. First, the T cells that recognize xenogeneic class I antigen are or become T killers (Fig. 1), and second, their function is blocked by anti-Leu-2a (the human counterpart of the mouse Lyt-2) (Fig. 4). Conversely, the T cells that recognize xenogeneic class II antigen are or become committed to IL-2 production (Fig. 2) and their induction is blocked by anti-Leu-3a (the marker found on the human helper T cell subpopulation) (Fig. 5). This shows the same correlation of blocking specificity and class specificity that has been seen in the human anti-human response (7, 10–12).

What can one conclude from these observations? The salient features of T cell recognition of antigen have been apparent for a number of years. These features, which are not predicted by a simple model based on antibody specificity, have led to a number of hypotheses for the structure of the T cell receptor or receptors but remain to be satisfactorily explained. They are listed here in a form that will be relevant to our subsequent discussion.

(a) All T cells recognize MHC (the fact that there may be some T cells that recognize free antigen does not undermine the significance of this as one can assume that they may do so by an alternative mechanism).

(b) MHC recognition has two features. First, it is allele specific—MHC-restricted

reactions to a nominal antigen X rarely, if ever, show cross-reactivity to X plus other MHC alleles (18). Thus, the polymorphic parts of the molecule seem most significant. Second, there is a recognition of class I MHC as distinct from class II. This is demonstrated by the fact that separate populations of cells (distinguishable by Lyt or Leu blocking experiments) recognize class I or class II MHC gene products. This recognition works across species (this paper).

(c) There is a strong bias (not absolute) associating the class I, Lyt-2$^+$ subset with killing and the class II, Lyt-2$^-$ subset with helper function.

(d) T cells that recognize a self-allele of MHC plus non-MHC antigen, X, have a high frequency of cross-reaction on foreign MHC alleles (19, 20).

We feel that the features listed in b above are best explained conceptually by two levels of recognition. In the first level of recognition, the T cell receptor must engage with an MHC molecule. It seems likely that the distinction between class I and class II is made here. The data presented in this paper suggest that the parts of the MHC molecule involved in this recognition are nonpolymorphic and must be sufficiently conserved so that the human T cell can effectively identify the mouse class I and class II components.

In the second level of recognition the T cell receptor must recognize the polymorphic part of the MHC molecule. The data presented here and those previously reported by Lindahl and Bach (15, 16) show that the capacity to recognize mouse alleles is contained within the human T cell repertoire.

In the classic two-receptor model, this second level of recognition would require two receptors, one for self-MHC and the other for the non-MHC antigen. In the altered-self model the polymorphic self-MHC structure plus the antigen must represent an overlapping collection of antigens that cross-react with the polymorphic structures of allogeneic cells and also presumably of xenogeneic MHC.

How is the concept of two levels of MHC recognition carried out in reality? We do not know. It is possible that the distinction between class I and class II recognition is achieved at the repertoire level and that there is one T cell repertoire for the allele-specific determinants of class I antigen and another for the allele-specific determinants of class II. In this case we would have to argue, for example, that the polymorphic variation of class I in the mouse could be recognized by the class I repertoire of the
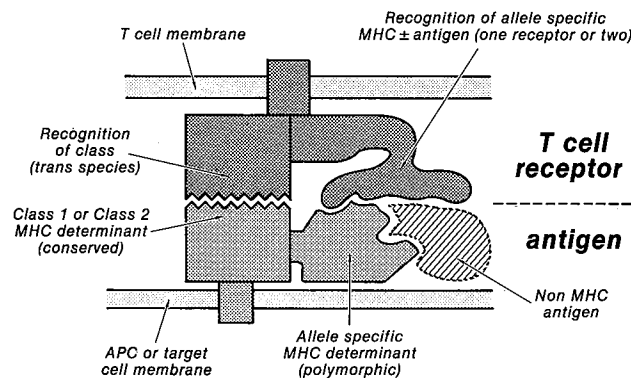

Fig. 6. Hypothetical model for the separate recognition of the invariant class determinants and the polymorphic variation (for discussion see text) of MHC.

human. It seems more likely that the recognition of class I and class II is clearly separated from the recognition of the MHC polymorphism. This is illustrated, conceptually, in Fig. 6. In the figure the different components of the T cell receptor and the MHC molecule are indications of a separation of functions and do not necessarily imply separate domains or separate gene products. In the figure it is suggested that recognition of the class epitope positions and restricts what the element recognizing the allele-specific epitope can react with. In this version of the model the only structure that the T cell receptor can engage is that part of the MHC molecule that carries the polymorphic variation and hence provides an explanation of the restriction of the T cell repertoire to these epitopes. The question of whether self-MHC plus X and allogeneic MHC are recognized by one recognition structure or two is not addressed by this model, although it is drawn in the single-recognition mode.

The blocking of class I recognition by anti-Lyt-2 and anti-Leu-2a reagents and the blocking of class II recognition by anti-Leu-2b suggests the possibility that these antigens are carried by or are spatially close to the class-recognizing structures (6, 12), but other possible explanations for the blocking data are also possible. The studies of Dialynas et al. (21), which show a strong correlation between loss of Lyt-2 expression and loss of non–lectin-mediated cytotoxicity in mutant cell lines, are also compatible with this concept.

In summary, the specificity for the allele-specific determinants in the T cell response to xenogeneic lymphocytes on the one hand, coupled with the ability to make a functional distinction between xenogeneic class I and class II structures on the other, argues for a model in which these two components of T cell recognition involve separate interaction sites on the T cell receptor.

Summary

Human T cells respond strongly to mouse major histocompatibility complex (MHC) antigens. The response is directed predominantly to the polymorphic determinants of the MHC antigens and there is little or no response to the nonpolymorphic determinants or to non-MHC antigens. Human cytotoxic T lymphocytes (CTL) are generated specific for the mouse class I MHC antigens and the CTL effectors are blocked by anti-Leu-2a antisera. Human interleukin 2-producing T cells are generated specific for mouse class II antigens and their induction is blocked by anti-Leu-3a antisera. These and other considerations lead us to propose a model for the T cell receptor that provides an explanation for several of the features of T cell recognition. In this model, the recognition of the "class" (I or II) of MHC antigen is separate from the recognition of the polymorphic determinants. We suggest that the initial recognition of the conserved "class" determinants positions another domain of the receptor so that it can only engage with the part of the MHC molecule carrying the polymorphic determinants.

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