Allorecognition of DR1 by T cells from a DR4/DRw13 responder mimics self-restricted recognition of endogenous peptides

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ABSTRACT   Coculture of a series of anti-DR1Dw1 allore-active human T-cell clones with autologous (DR4Dw4/DRw13Dw19) antigen-presenting cells and a series of recall antigens revealed that two of four clones tested proliferated in response to *Candida albicans*. One was restricted by DR4Dw4 and the other was restricted by DRw13Dw19. These results provide further evidence that many alloreactive T cells have a primary self-restricted specificity and cross-react on allogeneic major histocompatibility complex products. Structural comparison of the responder and stimulator DR molecules for these clones revealed that the regions predicted to contact the T cells' receptor, and thereby to determine self-restriction, are identical in sequence for DR4Dw4 and DR1Dw1 and differ by one residue between DRw13Dw19 and DR1Dw1. The DRβ residues that differ between responder, DR4Dw4 and DR13Dw19, and stimulator, DR1Dw1, are predicted to contribute to antigen binding. This implies that these anti-DR1 T cells may be specific for endogenous peptides that are bound by DR1 and not by the responder DR products, seen in a self-restricted manner. These T-cell clones also cross-reacted on DR4Dw13 and DRw14Dw16 molecules and on a human/murine hybrid class II dimer DR1β/I-Eα. These reactions are discussed in terms of self-restricted peptide recognition. Thus these data suggest that in certain responder/stimulator combinations allorecognition may resemble self-restricted recognition of fragments of endogenous antigens that are bound by stimulator but not by responder major histocompatibility complex products.

Structural definition of the complex of antigen and major histocompatibility complex (MHC) product recognized by antigen-specific MHC-restricted T cells has advanced rapidly in the last few years. This has resulted from the application of the techniques of genetic manipulation to MHC genes (1), the use of synthetic peptides as antigen (2), direct binding studies between antigen and MHC molecules (3, 4), and solving the three-dimensional structure of the HLA-A2 molecule (5). In contrast, the molecular basis of allorecognition of foreign MHC products remains unclear. Formulating a structural model to account for allorecognition depends on whether or not the T-cell repertoire is thought to be positively selected for recognition of self MHC products. If no such selection occurs then T cells reactive to allogeneic MHC need no more explanation than that the germ-line T-cell receptor (TCR) repertoire is skewed toward the recognition of MHC products. If, on the other hand, the mature T-cell repertoire is selected to have affinity for the self MHC, as has been demonstrated in several separate experimental systems (6–9), then, at face value, responsiveness to foreign MHC molecules appears to violate the rules of self MHC restriction. The prevailing view is that allorecognition results from molecular mimicry, whereby an allogeneic MHC molecule can resemble a complex of the self MHC molecule with a nominal antigen. Examples of T-cell clones that display

cross-reactions of this kind have been described (10–12). However, the precise specificity of self-restricted T cells both for antigen and for the MHC molecule with which it is presented and the recognition of linear sequences, rather than of conformational determinants as seen by immunoglobulins, creates some difficulty in envisaging how such molecular mimicry might occur.

Several lines of evidence lend support to the concept that the ligand recognized by many alloreactive T cells is not the allogeneic MHC molecule alone, but rather a binary complex of foreign MHC and a bound endogenous peptide (13–16). The data suggest that this may apply to both class I and class II MHC allorecognition. Although this model helps to explain the high frequency of T cells responsive to a given allospecificity (17), because it envisages that a single MHC molecule can generate multiple different binary complexes by binding multiple different peptides, it leaves open important questions about how self-restricted T cells recognize a non-self MHC product. In the experiments described here we propose that in some responder/stimulator combinations the specificity of alloreactive T cells may resemble self-restricted recognition of an array of endogenous peptides that are bound by stimulator but not by donor MHC products. Thus in these allelic combinations allorecognition may adhere to the same rules that apply to antigen-specific responses.

MATERIALS AND METHODS

Cell Lines and Culture Media. The DR1-expressing L cells (.5–3.1) were generated by cotransfection with DRα and DR1β cDNA clones by using the standard calcium phosphate precipitation technique and were kindly provided by R. Sekaly and E. Long (Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases). The DR1β/I-Eα and I-Eβ^k/I-Eα-expressing L cells (LT10B and LT66) were generated as described (18). The L cells were maintained in culture in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) fetal calf serum containing either hypoxanthine/aminopterine/thymidine or mycophenolic acid/xanthine/hypoxanthine in 25-cm^2 tissue culture flasks and subcultured, after trypsin treatment, at a 1:10 dilution twice weekly. Epstein–Barr virus-transformed lymphoblastoid cell lines (LCLs) were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, penicillin at 100 units/ml, and streptomycin at 100 μg/ml in 25-cm^2 flasks and were regularly passaged.

Antigens. *Candida albicans* was used as a whole cell extract, prepared using a Dyno-Mill. This material was generously provided by I. McKenzie (London School of Hygiene and Tropical Medicine).

T-Cell Clones. T-cell clones were obtained from a DR4Dw4/DRw13Dw19 responder against DR1Dw1 stimula-

Abbreviations: MHC, major histocompatibility complex; LCL, Epstein–Barr virus-transformed lymphoblastoid cell line; PBMC, peripheral blood mononuclear cell; TCR, T-cell receptor.
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Table 1. Self-restricted recognition of *C. albicans* by anti-DR1 alloreactive T cell clones

Stimulator cell	DR(Dw)	Antigen	[³H]Thymidine incorporation into responder T-cell clones, cpm			
			G3	G8	G11	G12
ND	4 (4), w13 (19)	−	89 (34.8)	147 (55.7)	749 (13.6)	376 (40.9)
		+	329 (27.0)	48,167 (2.6)	7,674 (7.9)	256 (59.8)
RIL	w15 (2), 4 (4)	−	—	156 (13.5)	1,227 (8.3)	—
		+	—	58,756 (11.7)	763 (9.8)	—
CD	w15 (2), w13 (19)	−	—	168 (71.4)	674 (17.0)	—
		+	—	1,314 (15.1)	10,286 (3.4)	—
BH	w15 (2), 7 (−)	−	—	133 (40.6)	772 (4.8)	—
		+	—	456 (46.0)	589 (8.1)	—
NF	1 (1), −	−	70,079 (1.1)*	89,439 (3.4)	6,884 (2.0)	33,289 (6.2)

Approximately 10^4 T clone cells were co-cultured with 10^5 x-irradiated PBMCs in the presence of *C. albicans* extract at 30 μg/ml. After 48 hr cultures were pulse-labeled with [³H]thymidine and harvested 18 hr later. Proliferation is expressed as cpm; numbers in parentheses are % error of the mean for triplicate cultures.
*Response to 3 × 10^4 NF DR1-LCLs.

tor cells. They were derived as described (16). Briefly peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll/Hypaque density gradient centrifugation and primed in the presence of irradiated PBMCs. After 7 days, primed lymphoblasts were cloned by limiting dilution in the presence of recombinant interleukin 2 and a fresh antigenic challenge in Terasaki plates (Sterilin, Teddington, U.K.). The clones were maintained in culture by weekly stimulation with DR1Dw1 PBMCs and interleukin 2. Cells were used for functional assays 1 week after their last stimulation.

T-Cell Assay. T-cell clones (10^4 cells per well) were cultured in the presence of irradiated PBMCs (5 × 10^4 cells per well) or irradiated LCL cells (3 × 10^4 cells per well) or mitomycin-C-treated transfected L cells (3 × 10^4 cells per well) in flat-bottomed microtiter plates (Flow Laboratories) in a total volume of 200 μl. [³H]Thymidine at 1 μCi (1 Ci = 37 GBq) was added after 48 hr and the cultures were harvested onto glass fiber filters 18 hr later. Proliferation was measured as [³H]thymidine incorporation as determined by liquid scintillation spectroscopy. The results are expressed as cpm or Δcpm ± SD or % error of the mean for triplicate cultures, as indicated in the legends.

RESULTS AND DISCUSSION

Self-Restricted Recognition of *C. albicans* by Anti-DR1Dw1 Alloreactive T-Cell Clones. We have described (16) a panel of anti-DR1Dw1 alloreactive T-cell clones generated from a DR4Dw4/DRw13Dw19 individual who had been primed *in vivo* against DR1Dw1 cells 2 years prior to the present study. The specificity of four selected T-cell clones was further explored in the experiments reported here. Examples have been described of antigen-specific self-restricted murine T cells cross-reacting on cells expressing allogeneic MHC molecules in the absence of specific antigen. Here we report the results of testing these anti-DR1Dw1 clones for self-restricted recognition of several common recall antigens (soluble extract of *Mycobacterium tuberculosis*, Tetanus toxoid, influenza virus A Texas 1/77 and B Singapore, and *C. albicans* extract). Shown in Table 1 are the proliferative responses of four of the anti-DR1Dw1 clones against the original DR1Dw1 stimulator and against *C. albicans* presented by PBMCs of various DR types, including autologous PBMCs. In addition to responding to the DR1Dw1 allostimulator cells, clones G8 and G11 also give a self-restricted response to *C. albicans* that for G8 is restricted by DR4Dw4 and for G11 is restricted by DRw13Dw19. These data lend further support to the concept that the T-cell repertoire is positively selected for self-restricted recognition and that alloreactive cells have a primary self-restricted specificity and cross-react on allogeneic MHC.

Allorecognition of DR1Dw1 by DR4Dw4/DRw13Dw19 T Cells May Mimic Self-Restricted T-Cell Recognition. Having demonstrated a self-restricted response for two of four of the clones, the sequences and structure of the responder and stimulator DR molecules were compared. Based on the HLA-A2 crystal structure, a hypothetical model of the three-dimensional structure of MHC class II molecules has been proposed (19). Based on this model the locations of amino acids in the α-helical portions and in the floor of the antigen-binding groove that differ in DR4Dw4, DRw13Dw19, and DR1Dw1 are portrayed in a schematic vertical cross-section in Fig. 1. Sequences comprising the α-helical portion and the floor of the α_1 domains are conserved since DRα
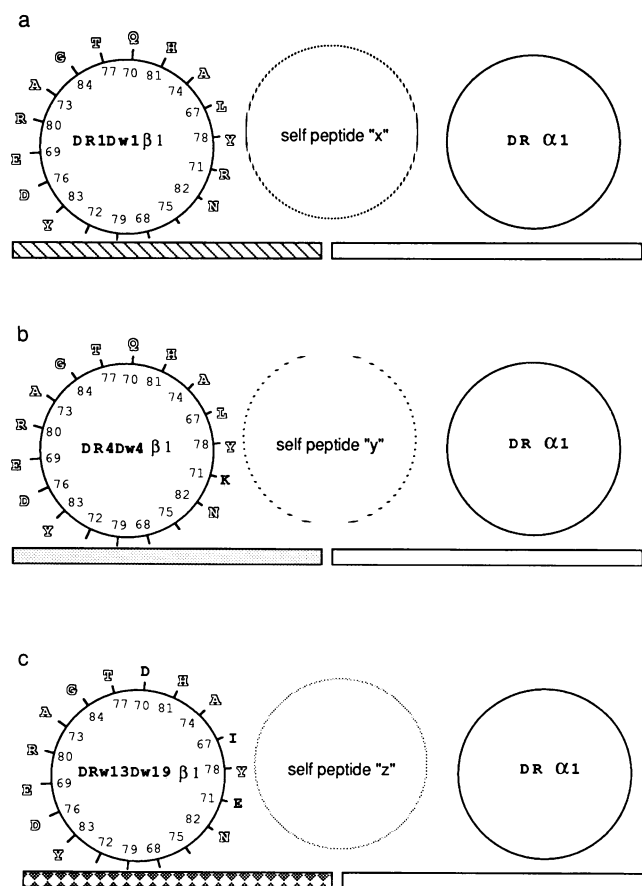

FIG. 1. Cross-section through the α-helices and the floor of anti-parallel strands that comprise the amino-terminal domains of DR1Dw1 (*a*), DR4Dw4 (*b*), and DRw13Dw19 (*c*). Sequences were derived from the following sources: DR1Dw1 (20), DR4Dw4 (21), and DRw13Dw19 (Jack Gorski, personal communication).

Table 2.   Cross-reactive recognition of DR4 and DR6 subtypes by alloreactive anti-DR1 T cell clones

| | | | [³H]Thymidine incorporation into responder T-cell clones, Δcpm | | | | | | | |
LCL	DR	Dw	G3		G8		G11		G12	
WT100BIS	1	1	69,911	(1.1)	62,715	(1.8)	35,311	(12.0)	69,885	(5.7)
BOLETH	4	4	119	(1.3)	474	(2.5)	−48	(4.0)	3	(5.1)
YAR	4	10	−142	(6.2)	281	(7.3)	−95	(6.1)	116	(12.1)
JHAF	4	13	−500	(3.3)	14,450	(6.5)	9,787	(3.2)	−320	(6.4)
PE 117	4	14	192	(3.7)	260	(14.9)	−39	(17.9)	−74	(0.5)
WDV	w13	18	40	(10.8)	185	(45.5)	−28	(12.9)	NT	
TEM	w14	9	456	(0.5)	840	(13.6)	245	(12.8)	497	(4.7)
AMALA	w14	16	10,318	(10.3)	56,090	(6.6)	−403	(4.9)	230	(41.4)

Approximately 10^4 T clone cells were cocultured with 3×10^4 x-irradiated homozygous LCLs. After 48 hr, cultures were pulsed-labeled with [³H]thymidine and harvested 18 hr later. Proliferation is recorded as Δcpm; numbers in parentheses are % error of the mean for triplicate cultures. NT, not tested. Homozygous LCL expressing DRw15 (Dw2 and Dw12), DRw17 (Dw3), DRw18 (Dw new), DRw11 (Dw5), and DRw8 (Dw8.1 and Dw8.3) were also tested and induced no proliferation.

shows no allelic polymorphism. Comparing DR4Dw4 and DR1Dw1, it is striking that, in the β_1 domains, all but one of the differences are located in the floor of the groove, as represented by the cross-hatching in DR1Dw1. Furthermore, the one residue that differs in the α-helix, position 71 (lysine for arginine), is predicted to point into the antigen-binding groove and is unlikely to make direct contact with the TCR. When DRw13Dw19 and DR1Dw1 are compared, there is one residue located on the upper face of the α-helix that differs, at position 70, glutamine (DR1Dw1) or aspartic acid (DRw13Dw19). Residues at two additional positions, 67 and 71, in the β_1 α-helix also differ between these β chains. Both of these positions are predicted to be located on the inner wall of the α-helix, although the residue at position 67 may be available for TCR contact. The change from leucine to isoleucine at 67 is, however, conservative. Again the residues comprising the floor of the groove show multiple differences from both DR1Dw1 and DR4Dw4. The consequences of the location of these sequence differences are, first, that the portions of the amino-terminal domains that are predicted to contact the T cell's receptor (i.e., the histotopic residues) are identical between DR4Dw4 and DR1Dw1 and differ by only one residue from DRw13Dw19. This enables allorecognition of DR1Dw1 to mimic self-restricted recognition by DR4Dw4- and DRw13Dw19-restricted T cells, such as G8 and G11, respectively (see Table 1). A second conclusion derived from this model is that DR1Dw1, DR4Dw4, and DRw13Dw19, although histotopically very similar, differ extensively in residues in the floor of the groove that are predicted to contribute to the antigen-binding region, or desetope, of the two β_1 domains. It follows that the antigen-binding grooves of these three types of DR molecule are likely to be occupied with different, although overlapping (22), sets of peptides derived from endogenous proteins. Thus these observations imply that allorecognition of DR1Dw1 by DR4Dw4- or

DRw13Dw19-restricted T cells can be regarded as self-restricted recognition of a series of peptides derived from endogenous proteins that are bound by DR1Dw1 and not by DR4Dw4 or by DRw13Dw19.

Structural Basis of the Patterns of β-Chain Cross-Reactivity of Anti-DR1Dw1 T-Cell Clones. To further explore the structural basis of allorecognition by these T-cell clones, their responses were measured to a panel of human homozygous LCLs expressing all the major DR types (these cells were chosen from the cell panel that was studied for the 10th International Histocompatibility Workshop). Selected results are shown in Table 2; no proliferation was detected when the clones were cocultured with cells expressing any of the DR types not shown in Table 2. As can be seen each clone exhibits a different pattern of cross-reactivity. Clone G12 is entirely DR1Dw1-specific, and the other three cross-react on the Dw13 subtype of DR4 and/or the Dw16 subtype of DRw14. The amino acid sequences of the β_1 domains of the relevant DRβ alleles are displayed in Fig. 2. As can be seen, the sequence that comprises the α-helical regions of DR1Dw1 and DRw14Dw16 (i.e., residues 52–86) are identical. Thus the cross-reaction on DRw14Dw16 by the DR4Dw4-restricted clone G8 may further reflect the similarity of this alloresponse to self-restricted recognition. The presumed capability of DRw13Dw16 to bind and present the same endogenous peptides to clones G8 and G3 as are bound by DR1Dw1 may be conferred by two shared residues. The first is Arg-71 that is predicted to point into the antigen-binding groove; the second is Glu–28, a residue in the floor of the groove that plays a key role in the binding of cytochrome peptides by I-E-restricted mouse T cells (24). The cross-reaction of G8 on DR4Dw13 may be best accounted for by the Ala → Glu change at position 74, which is unique to this DR4 subtype. Given the identity of DR4Dw13 to DR4Dw14, which fails to stimulate at every other position, it may be that the

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           1        10        20        30        40        50        60        70        80        90
DR1  Dw1    GDTRPRFLWQLKFECHFFNGTERVRLLERCIYNQEESVRFDSDVGEYRAVTELGRPDAEYWNSQKDLLEQRRAAVDTYCRHNYGVGESFTVQRR

DR4  Dw4    --------E-V-H------------F-D-YF-H---Y-----------------------------------K-----------------------
DR4  Dw10   --------E-V-H------------F-D-YF-H---Y-----------------------------I--DE--------------V--------
DR4  Dw13   --------E-V-H------------F-D-YF-H---Y---------------------------------E-----------V--------
DR4  Dw14   --------E-V-H------------F-D-YF-H---Y-------------------------------------------V--------

DRw13 Dw18  --------EYSTS-----------F-D-YFH----N---------F-----------------I--DE--------------V--------
DRw13 Dw19  --------EYSTS-----------F-D-YFH----N---------F-----------------I--DE--------------------
DRw14 Dw9   --------EYSTS-----------F-D-YFH----F-----------------A-DH---------R---E-----------V--------
DRw14 Dw16  --------EYSTS-----------F---YFH----N---------------------------------------------------
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FIG. 2.   Amino acid sequences of the β_1 domain of DR1Dw1 and DR4 and DR6 subtypes. Sequences are shown using the one-letter amino acid code and are derived from the following sources: DR4Dw10 (21), DR4Dw13 (21), DR4Dw14 (21), DRw14Dw9 (23), DRw14Dw16 (27), and DRw13Dw18 (23).
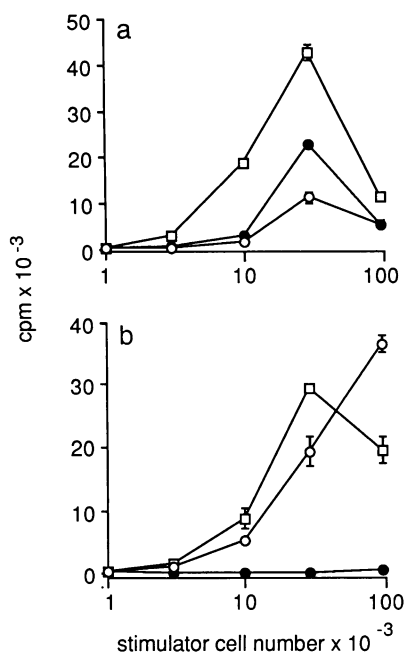
FIG. 3.   Proliferative responses of DR1-specific alloreactive T-cell clones (G8 and G11) to various numbers of LCLs are shown as cpm × 10^{-3}, with standard deviations. Allostimulator cells were as follows: irradiated DR1Dw1 LCL (WT100BIS) (□), DR4Dw13 LCL (JHAF) (○), and DR6Dw16 LCL (AMALA) (●). After 48 hr, cultures were pulse-labeled with [^{3}H]thymidine and harvested 18 hr later. (*a*) Clone G8. (*b*) Clone G11.

Glu-74 enables DR4Dw13 to bind some of the same endogenous peptide(s) that bind to residues in the floor of DR1Dw1. The comparable efficiency of DRw14Dw16- to DR1Dw1-expressing cells and the lower efficiency of cells expressing DR4Dw13 in stimulating this DR4Dw4-restricted cell, as shown in Fig. 3, might be predicted from the sequence comparisons. All the LCLs used in the dose–response comparison expressed indistinguishable levels of HLA-DR, as judged by flow cytometric analysis (data not shown). Clone G11, which is DRw13Dw19-restricted in response to *C. albicans*, also cross-reacts on DR4Dw13 cells but there is no response to cells expressing DRw14Dw16. It is clear, therefore, that residues in the floor of the groove must contribute to this alloresponse, since DRw14Dw16 is identical to DR1Dw1 in the α-helix. Again it may be that Glu-74 in DR4Dw13 may provide an alternative means of binding the same peptide(s) that bind to DR1Dw1.

Substitution of I-Eα for DRα Has a Variable Effect on DR1Dw1 Allorecognition. The contribution of the DRα chain to DR1Dw1 allorecognition by these clones was examined using transfected murine L cells expressing either DR1β/DRα or DR1β/I-Eα. The results are presented in Table 3. We

have shown that all the clones presented here are able to respond to DR1 expressed on L cells (16). Additional results implied that the transfected L cells were internalizing, processing, and presenting peptides derived from human proteins that were shed or secreted by co-cultured human cells (16). Transfectants were then prepared that express the same DR1β chain, but paired to the murine I-Eα polypeptide. As can be seen two clones, G3 and G11, responded to cells expressing DR1β with either DRα or I-Eα. In contrast G12 gave no proliferative response to the DR1β/I-Eα transfectant, and the response of G8 was only just above background. Species-mismatched MHC class II dimers comprising α and β chains of the human and murine homologous loci, DR and I-E, are assembled and expressed at the cell surface very efficiently after cotransfection (R.I.L., unpublished observations). In addition, two influenza virus-specific DR1-restricted human T-cell clones responded equally well to transfectants expressing DR1β/DRα or DR1β/I-Eα (18). These observations suggest that there is a great deal of similarity between these two α chains in functionally important regions. According to the class I-derived model of class II structure, the α chains of these two loci show extensive sequence similarity in the floor of the groove and through most of the α-helical stretch. This is shown schematically in Fig. 4. Most of the differences would be predicted to fall in the loops joining the antiparallel strands and at either end of the α-helix. There is one substitution in the exposed part of the floor of these domains at position 6 (leucine in Eα for asparagine in DRα).

There are three possible ways to account for the absent response of clone G12 and the minimal response of clone G8 to the DR1β/I-Eα transfectant. The Asn → Leu change at position 6 or one of the changes at either end of the α_1 α-helix may interfere with the binding of a peptide(s) for which these clones are specific. Alternatively one of the altered residues at either end of the α-helix that points up, toward the TCR, may substantially lower the affinity of the TCR–MHC interaction. It is also conceivable that any of these substitutions may have an indirect effect by altering the conformation of the α_1 domain locally or distantly. The third possible explanation for the loss of recognition due to I-Eα substitution is that the peptide corecognized by these clones with DR1 is a processed fragment of the $DR\alpha_1$ domain derived from one of the regions in which I-E sequence is significantly different. This last, remote, possibility can be tested formally by the addition of the appropriate peptides. All these interpretations have to be taken in the context of ignorance about the contribution of the invariant DRα and I-Eα chains to T-cell recognition. Nonetheless, the reactivity patterns of these anti-DR1 clones imply a key role for residues in the $DR\alpha_1$ domain.

The results of this analysis suggest that in certain responder–stimulator combinations recognition of foreign

Table 3.   Recognition of transfected L cells expressing DR1β/I-Eα by anti-DR1 alloreactive T cell clones

	[^{3}H]Thymidine incorporation into responder T-cell clones, Δcpm			
Stimulator cell	G3	G8	G11	G12
L cell	223 (75.4)	254 (39.5)	155 (14.8)	−53 (8.9)
DR1β/DRα L cell	28,866 (12.8)	23,433 (12.9)	19,014 (19.6)	12,692 (20.9)
DR1β/I-Eα L cell	24,706 (1.6)	1,309 (12.6)	5,875 (4.9)	−32 (16.7)
I-Eβ^k/I-Eα L cell	−14 (17.6)	72 (45.5)	143 (30.3)	32 (57.8)
DR1 LCL	30,873 (0.8)	27,290 (15.7)	8,586 (35.4)	15,022 (11.0)

Approximately 10^4 T clone cells were cocultured with 3 × 10^4 x-irradiated homozygous LCL or mitomycin-treated transfected murine L cells expressing the species-mismatched MHC class II dimer DR1β/I-Eα. After 48 hr, cultures were pulse-labeled with [^{3}H]thymidine and harvested 18 hr later. Proliferation is recorded as Δcpm; numbers in parentheses are % error of the mean for triplicate cultures.

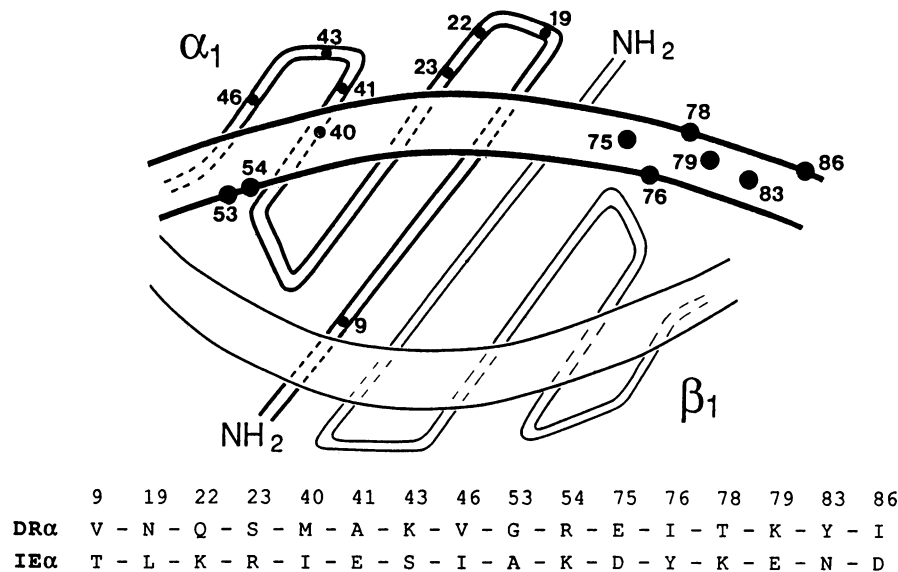|	9	19	22	23	40	41	43	46	53	54	75	76	78	79	83	86
DRα	V	N	Q	S	M	A	K	V	G	R	E	I	T	K	Y	I
IEα	T	L	K	R	I	E	S	I	A	K	D	Y	K	E	N	D

FIG. 4. Structure of the amino-terminal domains of MHC class II molecules are shown. The α_1 domain is highlighted. The α-helical portions are shown as broad bands overlying the anti-parallel strands beneath. Residues that differ between DRα and I-Eα are shown as solid circles in their predicted positions. The amino acids found at these positions are displayed below using the single-letter code. The sequences were derived from the following sources: DRα (25) and I-Eα (26).

MHC products may be very similar to the self-restricted recognition of nominal antigen. This can be envisaged when the histotopic TCR-contacting surfaces of the relevant responder and stimulator MHC molecules are very similar, and the putative antigen-binding residues are sufficiently different to enable the stimulator cells to display peptides derived from endogenous proteins that have not been encountered previously by responder cells. One of the implications of this interpretation of allorecognition is that the molecular basis of recognition of foreign MHC products may be heterogeneous. When the histotopic surfaces of responder and stimulator MHCs differ extensively, allorecognition may diverge widely from self-restricted recognition. A prediction that arises from these proposals is that the precursor frequencies of alloreactive cells may vary substantially between different donor/stimulator combinations. This will be determined by the distribution of structural similarities and differences between responder and stimulator in the regions of the MHC molecules involved in restricted recognition and in the binding of antigen. Paradoxically the highest precursor frequencies may be found in responder/stimulator combinations that are histotopically similar. This has potential relevance to the outcome of tissue transplantation.

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