Contribution of Direct and Indirect Recognition Pathways to T Cell Alloreactivity

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Summary

T cells from an HLA-DR11/DR12 responder were stimulated in mixed lymphocyte culture with cells carrying the DR1 antigen. After priming, T cells proliferated in response to both DR1-positive-stimulating cells and a peptide derived from a polymorphic region of the HLA-DRβ1*0101 chain presented by responder's antigen-presenting cells (APC). The dominant epitope recognized by the primed T cells corresponded to residue 21–42 and was presented by the responder's HLA-DR12 antigen. The DR1 peptide–reactive T cells express T cell receptor Vβ3. The results demonstrate that allopeptides derived from the processing and presentation of donor major histocompatibility complex molecules by host-derived APC trigger alloreactivity. The frequency of T cells engaged in the indirect pathway of allorecognition is about 100-fold lower than that of T cells participating in the direct recognition of native HLA-DR antigen. However, indirect allorecognition may play an important role in chronic allograft rejection, a phenomenon that is mediated by the activation of T helper cells and of alloantibody-producing B cells.

Two pathways of antigen recognition have been considered in T cell responses to MHC alloantigens (1–4). A direct pathway involves T cells capable of recognizing alloantigens as intact molecules on the surface of allogeneic stimulator cells. The TCRs recognize, in this case, unknown peptides bound in the groove of allogeneic MHC molecules and/or adjacent epitopes of the allogeneic MHC molecule. The precursor frequency of T cells involved in the direct recognition pathway is extremely high, with estimates of 1–5% of T cells exhibiting blastogenic responses to allogeneic-stimulating cells in MLC (2). There is ample evidence that the direct pathway of allorecognition is the principal contributor to antigraft cytotoxic T cell responses mediating early rejection episodes. The very high number of precursor T cells participating in direct allorecognition has been attributed to molecular mimicry resulting from the engagement of TCRs whose innate reactivity was for a complex formed by a self-MHC molecule with an endogenous or exogenous peptide (5–9).

The indirect pathways of allorecognition has come into focus more recently, with the realization that this pathway may explain T helper cell–dependent cytotoxic T cell and alloantibody responses (10–13). In this pathway, T cells recognize graft MHC alloantigens that have been processed and presented by host APC. Indirect recognition is restricted by the host MHC class II molecule, which has bound a peptide derived from the processing of an allogeneic MHC molecule that is, therefore, the classical pathway of conventional antigen recognition by CD4 T cells (10–16). The involvement of alloantigen-specific CD4 T helper cells, as mediators of alloantibody generation, suggests that the indirect pathway plays an essential role in chronic rejection, e.g., in the steady but continuous attrition (2–5%/yr) of organ allografts late after transplantation (17, 18).

In previous studies we have shown that synthetic peptides derived from the amino acid sequence of the DRβ1*0101 chain stimulate the reactivity of T cells from allogeneic (DR11) and syngeneic (DR1) responders (13).

The aim of this study was to establish the relative contribution to alloreactivity of the direct and indirect pathways of T cell recognition of an allogeneic MHC class II molecule. T cells from an HLA-DR11/12 responder were primed in MLC with allogeneic DR1-positive cells and then tested for reactivity to DR1 stimulators and to synthetic DR1 peptides in the presence of responder's APC. We now report that T cells involved in indirect recognition are 100-fold less frequent than T cells participating in the direct recognition pathway, and that the dominant epitope that they recognize in the context of DRβ1*1201 lies within residue 21–42 of the DRβ1 chain.

Materials and Methods

HLA Typing. The HLA class II genotype of all PBMC selected for these experiments was characterized by conventional serology and by genomic typing of in vitro amplified DNA with sequence-

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specific oligonucleotide probes (SSOP) for DRβ1, using PCR primers and SSOP provided by the XI International Histocompatibility Workshop (19).

Peptide Synthesis. Peptides were synthesized with an automated peptide synthesizer (430A; Applied Biosystems, Inc., Foster City, CA), using modified Merrifield chemistry, as previously described (13, 20, 21). Seven partially overlapping peptides, corresponding to residues 1–20, 11–30, 21–42, 31–50, 43–62, 51–70, and 66–90, were synthesized.

Limiting Dilution Analysis. Responding cells were obtained from the peripheral blood of a healthy male (LS) with the HLA-DR11, DQ3/DR12, DQ3 genotype. PBMC at 10^6/ml were stimulated in 24-well plates (Costar Corp., Cambridge, MA) with an equal number of irradiated (3,000 rad) PBMC from an individual (EC) whose genotype is DR1, DQ1/DR3, DQ2, in RPMI 1640 supplemented with 10% pooled human serum, 2 mM L-glutamine, and 50 μg/ml gentamicen (Gibco Laboratories, Grand Island, NY) 11 d after MLC stimulation T cells were tested in an limiting dilution assay (LDA),[1] e.g., at concentrations of 2 × 10^4 to 50 cells/well for reactivity to: (*a*) irradiated autologous (LS) APC (5 × 10^4 PBMC/well); (*b*) irradiated allogeneic PBMC (5 × 10^4/well) from a DR1, DQ1 homozygous stimulator; (*c*) irradiated allogeneic PBMC from an individual homozygous for DR2, DQ1, e.g., matching the stimulator for DQ1; (*d*) autologous APC (5 × 10^4/well) plus a cocktail of seven different synthetic 20-mer peptides spanning the first domain of DRβ1*0101; and (*e*) irradiated L cells transfected with HLA-DR1 (XIth International Histocompatibility Workshop). All cultures were fed after 3 d with fresh medium containing rIL-2 (5 U/ml). On day 6, cultures were labeled with [^{3}H]TdR and harvested after 18 h. The precursor frequency of reactive T cells was calculated as described (13, 20).

Establishment of Allopeptide-Specific T Cell Line and Clones. T cells from individual LS, which were stimulated for 11 d in 1°MLC with irradiated PBMC from EC, were primed in 24-well plates at 10^6/ml with 10 μg/ml each of the seven HLA-DR1 peptides in culture medium. 3 d after stimulation rIL-2 (Boehringer Mannheim Biochemicals, Indianapolis, IN) was added at 5 U/ml. The cultures were fed every 3 to 4 days with medium containing rIL-2 at 20 U/ml. After 14 d, T cells (2 × 10^6/ml) were stimulated with the peptide mixture and irradiated autologous PBMC (2 × 10^6/ml) in medium containing 20 U/ml of rIL-2. The culture was restimulated two more times, at 14-d interval, under identical conditions. The resulting T cell line (TCL), named TCL-LS-anti-EC, was tested for reactivity to each of the seven peptides. This TCL was cloned by limiting dilution at 0.5 cells/well in medium containing DR1 peptides, irradiated autologous APC, and rIL-2. T cell clones (TCC) were expanded by restimulation with peptide and autologous APC.

Proliferation Studies. Responding T cells (2 × 10^4/well) were cocultured with 5 × 10^4 irradiated APC in round-bottomed microculture plates (Costar Corp.). DR1 peptides at 2.5 μg/ml and/or stimulating cells (5 × 10^4/culture) were added to the cultures. After 48 h of incubation the cultures were labeled with [^{3}H]thymidine and then harvested after an additional 18 h.

Antibody Blocking Assay. mAbs were added to the cultures at the initiation of the blastogenesis assay. L243 and W6/32 (American Type Culture Collection, Rockville, MD) were used as cell culture supernatants. Anti-DP, -DQ, -CD4 and -CD8 antibodies (Becton Dickinson & Co., Mountain View, CA) were dialyzed against medium and used at 1 μg/ml.

Determination of TCR-Vβ usage by PCR. cDNA was prepared from total RNA by reverse transcription and amplified by PCR using Vβ and Cβ primers as previously described (13, 21). The amplified products were separated on 2% agarose gel. 1 μg of HaeIII-digested ϕ x 174 DNA (GIBCO BRL, Gaithersburg, MD) was run in parallel as molecular weight markers.

Results

LDA of T Cells Participating in Direct and Indirect Allorecognition. We first tried to determine what is the relative contribution of direct and indirect recognition to an MLC response. For this, we primed T cells, in a primary MLC, to allogeneic DR1-positive cells and we measured in an LDA the frequency of cells responding to DR1-positive-stimulating cells and to synthetic DR1 peptides. Because the stimulating cells also expressed the possible target structures DQ, DP, and DR3, to discriminate between the response to DR1 and the response to the other MHC class II antigens, we used as stimulator cells that are homozygous for DR1, DQ1 and for DR2, DQ1. In addition, we used as stimulator L cells transfected only with DR1. The frequency of cells responding directly to DR1, DQ1 homozygous cells was 1:328 and that of cells reacting to the DR1 L cell transfectant was 1:361 (Table 1). The frequency of cells involved in the direct recognition of DQ1, as expressed by DR2, DQ1 homozygous cells, was 1:1,529. The 1°MLC-stimulated T cells showed no reactivity to DR1 peptides when tested in cultures without APC. However, when irradiated, autologous APC and the DR1 peptide mixture were added together, proliferation was observed. The estimated frequency of T cells capable of recognizing DR1 peptide(s) bound to an autologous MHC class II molecule was 1:43,992 (Table 1). Thus, the frequency of cells engaged in the indirect pathway of recognition is ~100-fold lower than that of cells engaged in direct recognition.

Proliferative Response of TCL to DR1 Peptides. The LDA results showed that T cells recognizing in context of self-MHC the processed allogeneic DR1 molecule, which was shed or secreted by allostimulating cells, were activated during 1°MLC. To determine the structure of the dominant epitope of the DR1 molecule that these cells recognized, we challenged them individually with each of the seven (partially overlapping) synthetic peptides derived from the amino acid sequence of the DRβ1*0101 molecule.

Blastogenic responses occurred only when the DR1 peptide 21–42 was added to the cultures (Fig. 1). None of the other synthetic DR1 peptides restimulated the cells in the presence of the responder's APC. Hence, peptide 21–42 comprises the dominant epitope of the DR1 molecule.

MHC Restriction Studies. Having determined that the DR1 peptide 21–42 comprises the dominant DR1 epitope recognized by TCL-LS-anti-EC, we next tried to identify the MHC restriction element. For this, TCL-LS-anti-EC and the TCC derived from it were tested for reactivity to peptide 21–42 in the presence of APC sharing with LS either the DRβ1*1101 or DRβ1*1201 allele. The responses of the TCL and of six

[1] *Abbreviations used in this paper:* LDA, limiting dilution analysis; TCC, T cell clones; TCL, T cell lines.

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Table 1. *Frequency of Reactive T Cells*

Stimulating cells	HLA-DR, DO phenotype	Reactive cells
Allogeneic PBMC (NN)	DR1, DQ1	1:328
Allogeneic PBMC (NS)	DR2, DQ1	1:1,529
L cell transfectants	DR1	1:361
Autologous PBMC (LS) + DR1 peptides	DR11, DQ3/ DR12, DQ3	1:43,992
Autologous PBMC (LS)		0

PBMC from responder LS were stimulated in 11-d MLC with irradiated PBMC from a DR1,DR1-positive donor. The MLC-primed T cells were tested in LDA for reactivity to DR1-positive cells and DR1 peptide.

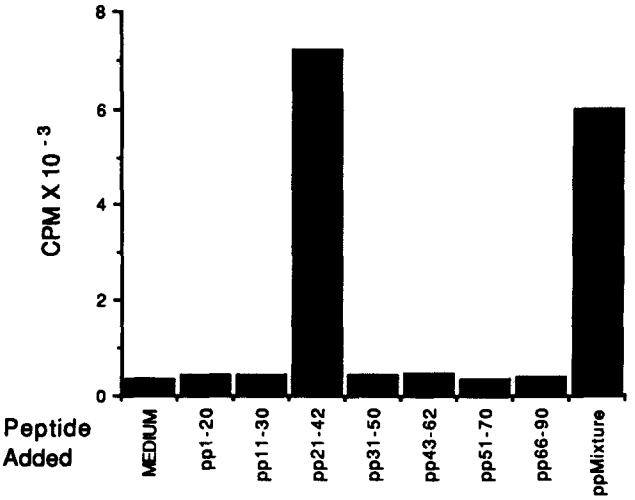

Figure 1. Determination of dominant epitope of DR1 molecule. TCL-LS-anti-EC (2×10^4 cells/well) was tested for reactivity to DR1 peptides corresponding to residues 1–20, 11–30, 21–42, 31–50, 43–62, 51–70, and 66–90 (2.5 μg/ml) in the presence of autologous APC (5×10^4 cells/culture).

representative clones are shown in Table 2. The line and the clones reacted to peptide 21–42 when DR1201-positive cells but not DR1101-positive cells were used as APC. The restrictive element used by the TCL and TCC for the recognition of DR1 peptide 21–42 is, therefore, the DR1201 molecule. mAbs specific for HLA-DR and CD4 inhibited the response of the TCL and TCC to peptide 21–42 presented by autologous APC (Fig. 2). There was no inhibition of proliferative responses by mAbs to HLA-class I, DQ, and DP, indicating that an HLA-DR molecule was solely responsible for the presentation of DR1 peptide 21–42.

Recognition of Native DR1 Molecule by DR1 Peptide-specific TCC. Native DR1 molecules, expressed on DR1-positive cells, elicited reactivity only when the responder's APC or APC from other individuals carrying the DRβ1*1201 allele were added to the cultures (Table 3). In the absence of such APC, the native DR1 molecule failed to stimulate, indicating that processing of the DR1 molecule and binding of the DR1 peptide corresponding to the dominant epitope to the DR1201 molecule are required for recognition to occur. We next explored the possibility that cells from individuals heterozygous for DR1 and DR1201 would display a similar complex on their surface. However, stimulating cells from an individual (NV) carrying both the restrictive element, DR12, and the

Table 2. *MHC Restriction of Peptide Recognition*

		[^{3}H]TdR incorporation													
	DRβ1 genotype	TCL		TCC 1.1		TCC 10.1		TCC 30.1		TCC 30.3		TCC 30.6		TCC 30.7	
Cell	of APC	–	+ Peptide	–	+ Peptide	–	+ Peptide	–	+ Peptide	–	+ Peptide	–	+ Peptide	–	+ Peptide
LS	1101/1201	129	50,752	83	30,270	1,356	54,929	111	55,883	1,180	51,017	1,437	42,330	230	32,845
PR	1101/07	64	81	45	43	110	163	52	50	48	55	87	23	36	33
NM	1104/0101	83	42	52	40	235	177	51	42	43	45	110	107	42	45
NV	1201/0101	353	50,867	271	28,366	1,148	51,664	596	56,461	529	43,455	964	41,254	367	34,157
RA	1201/1601	29	49,070	57	23,705	608	43,684	90	49,564	743	34,550	270	36,124	59	29,734
RB	1201/0408	1,388	50,317	2,311	38,186	14,409	52,579	2,546	46,257	2,377	37,159	9,385	37,330	2,059	37,027
RN	1501/07	112	71	60	35	357	99	54	44	113	52	162	79	49	40
FL	0301/1601	103	121	69	57	346	561	57	73	107	96	238	158	45	63
EC	0301/0101	131	83	70	37	219	199	50	59	160	61	107	82	37	30

TCL LS-anti-EC and TCC (2×10^4/well) were tested for reactivity to DR1 peptide 21–42 (2.5 μg/ml) in the presence of APC (5×10^4/well) carrying different DR alleles.
Reactions were set up in triplicates. SD is <10%.

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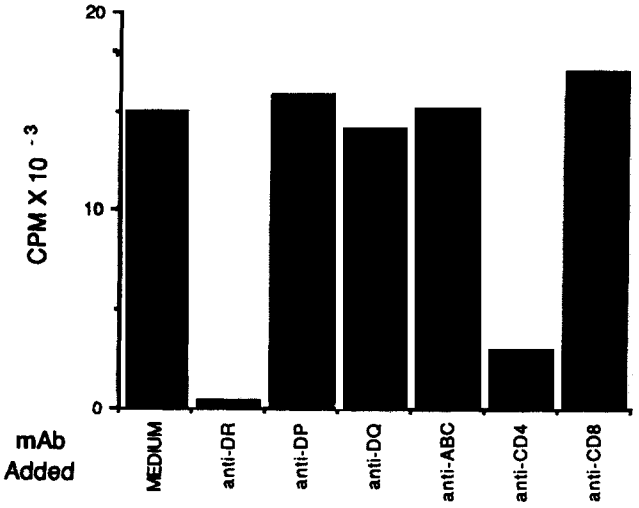

Figure 2. Effect of mAbs on the proliferative response of TCL-LS-anti-EC to the DR1 peptide 21–42. TCL-LS-anti-EC (2×10^4 cells/well) was cultured with autologous APC (5×10^4) and DR1 peptide 21–42 (2.5 μg/ml). mAbs were added at the initiation of the proliferative assay.

stimulatory molecule, DR1, elicited activation only when exogenous, synthetic peptide 21–42 was added to the cultures (Table 2). In cultures without exogenous peptide there was no reactivity, suggesting that the amount of DR1 peptide 21–42 presented by the DR12 molecule expressed by this stimulator was insufficient to trigger activation.

Molecular Mimicry of DR12-DR1 Peptide Complexes by DR4 Allelic Products. When PBMC expressing different MHC alleles were used for ascertaining the MHC-restrictive element required for TCC activation, an important and consistent exception was noted: cells from an individual carrying the DR4 allele, DRβ1*0408, stimulated the clones even without the addition of exogeneous DR1 peptide. In view of this observation we tested our entire panel of DR4 variants for their ability to stimulate the TCL and TCC. Cells expressing DRβ1*0401, 0403, and 0404 elicited strong reactivity. Cells carrying the DR4 alleles, DRβ1*0405, 0406, 0407, and 0408, had little stimulatory activity, while 0402-positive cells were not stimulatory (Table 4). Hence, TCC that recognize (indirectly) DR1 peptide 21–42 presented by the DR1201 molecule can also recognize directly products of certain DR4 alleles plus unknown peptides(s). The latter probably present a determinant with structural homology to the DR1201-DR1 peptide complex. This finding supports the notion that molecular mimicry accounts at least in part for direct recognition of allogeneic MHC molecules (9).

TCR-Vβ Gene Usage. In previous studies we have shown that TCL that recognize the DR1 peptide 21–42 in context of a self-MHC molecule, such as DRβ1*1101 and DRβ1*0101, have a limited TCR-Vβ gene usage (13, 21). In these studies, however, the TCL were generated by priming PBMC with the synthetic peptide. To establish whether indirect recognition of DR1 peptide, derived from the natural processing of native DR1 molecule, is also the function of a restricted number of TCR-Vβ families, we analyzed the TCR-Vβ genes expressed by TCL-LS-anti-EC and by the clones derived from it. The TCL and all the six TCC that were analyzed expressed Vβ3 (Fig. 3). This result is consistent with our previous finding that the TCR-Vβ gene usage, in alloreactive TCC involved in indirect recognition, is biased.

Discussion

Central to the problem of allograft rejection is the understanding of the molecular events resulting in allostimulation. The possibilities that the TCRs of some alloreactive cells bind directly to the allogeneic MHC molecule with or without a bound peptide, while other TCRs are engaged by complexes formed by self-MHC with peptides derived from an allogeneic MHC molecule, have been both substantiated (1–16).

Table 3. *Recognition of Naturally Processed DR1 Molecule by TCL and TCC*

Stimulator	HLA-DRβ1 genotype of (irradiated) PBMC	APC	[³H]TdR Incorporation						
			TCL	1.1	10.1	30.1	30.3	30.6	30.7
						mean cpm			
EC	0101/0301	LS 1101/1201	4,424	3,246	3,509	4,065	5,004	3,890	3,479
EC	0101/0301	RA 1201/1601	4,118	3,869	4,376	4,453	4,260	3,602	3,255
EC	0101/0301	RV 1501/07	237	210	300	262	135	235	322
MN	0101/1104	LS 1101/1201	6,014	3,105	4,002	4,380	4,410	3,435	2,946
FL	0301/1601	LS 1101/1201	209	339	144	204	279	303	311
RV	1501/07	LS 1101/1201	359	267	144	263	237	355	254

TCL-LS-anti-EC and TCC (2×10^4/well) were stimulated with irradiated DR1-positive and -negative (control) PBMC (5×10^4/well) in the presence of autologous (LS) and hemiallogeneic APC (5×10^4/well). Cultures were labeled after 48 h and harvested 18 h later.

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Table 4. *Recognition of DR4 by Peptide-specific TCL and TCC [³H]TdR incorporation*

DRβ1 genotype of APC		[³H]TdR incorporation													
		TCL		TCC 1.1		TCC 30.1		TCC 30.3		TCC 30.6		TCC 30.6		TCC 30.7	
		−	+ Peptide	−	+ Peptide	−	+ Peptide	−	+ Peptide	−	+ Peptide	−	+ Peptide	−	+ Peptide
		mean cpm													
PM	0401/1501	4,491	2,505	8,299	7,907	23,596	24,088	4,859	3,724	8,093	7,408	19,649	17,439	4,977	3,989
SS	0402/1502	157	329	45	41	454	192	71	37	78	62	133	154	47	45
LD	0403/0101	15,588	11,342	8,105	6,401	32,374	21,231	14,750	10,607	12,870	10,993	25,497	22,174	9,780	7,637
SD	0404/0301	16,269	17,360	14,331	17,687	33,742	33,873	15,672	17,264	18,502	18,977	25,503	26,581	13,887	12,488
DY	0405/1201	1,639	51,410	1,055	28,305	11,809	42,299	2,542	40,394	3,024	37,437	6,336	33,345	1,718	32,553
ON	0406/1402	2,173	931	2,010	963	11,646	5,987	5,791	2,003	2,851	1,429	8,780	5,095	2,118	1,545
MN	0407/1402	636	687	1,242	420	5,399	3,993	1,393	276	1,534	705	1,910	2,312	1,123	601
RB	−0408/1201	1,398	50,317	2,311	38,186	11,469	52,579	2,546	46,217	2,377	37,159	9,385	37,330	2,059	37,027

TCL and TCC (2×10^4/well) were tested for reactivity to irradiated PBMC (5×10^4/well) from individuals heterozygous for different HLA-DR-4 alleles in the presence or absence of DR1 peptide 21–42.

The direct recognition pathway accounts most likely for the vigorous immune response elicited by allogeneic tissue and organs early after transplantation (10, 12). This early reaction may result in acute rejection, which can be suppressed by timely and vigorous therapy with steroids, OKT3, and/or increased dosage of immunosuppressants. Donor dendritic cells are suspected to be the major source of MHC class II antigens that stimulate T helper reactivity and subsequently contribute to the activation of cytotoxic CD8 effectors.

For most organ allograft systems the major threat to long-

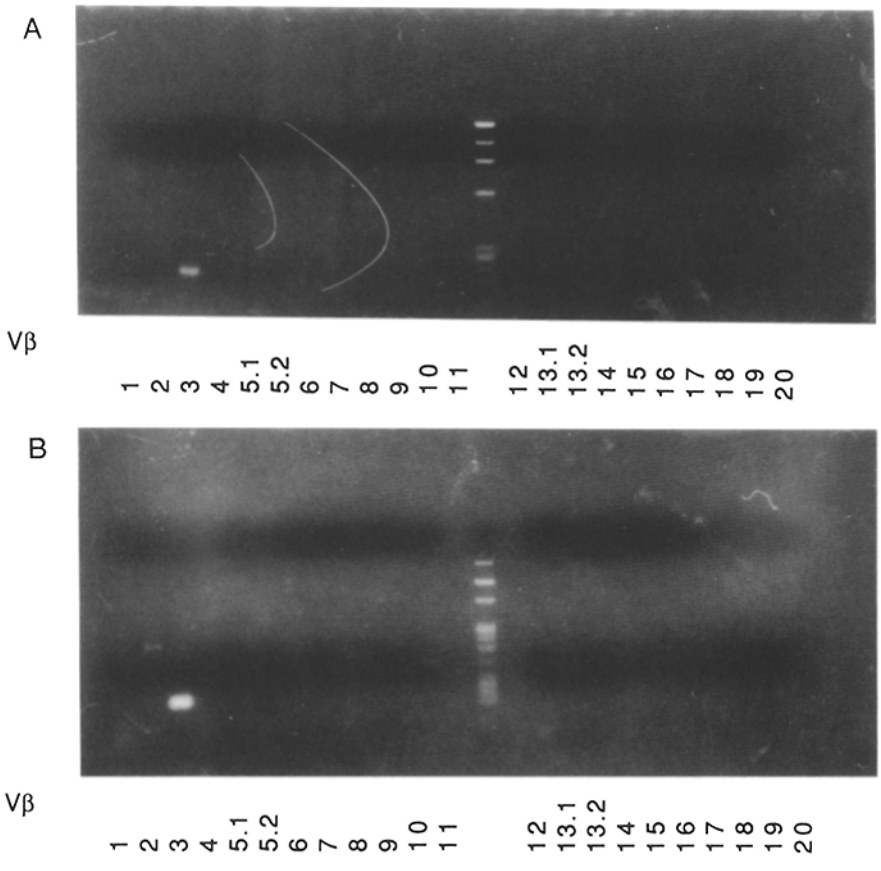

Figure 3. Amplification of cDNA from TCL-LS-anti-EC and TCC 1.1. RNAs from TCL (*A*) and TCC (*B*) were reverse transcribed, PCR amplified, and subjected to electrophoresis on 2% agarose gel. 1 μg HaeII-digested φ x 174 DNA was run in parallel as molecular weight markers.

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term survival remains chronic rejection, a slow and insidious process that often takes years for completion (17, 18). Chronic rejection has been associated with the production of lymphokines and cytokines damaging the intima of the vessels and inducing the proliferation and differentiation of alloantibody-producing B cells (17, 18). Because the alloantibodies formed during chronic rejection react with donor cells and often exhibit antidonor MHC specificity, this process is likely to be mediated by T helper cells recognizing MHC peptides derived from the donor MHC molecules and bound to host MHC molecules (13). Donor alloantigens, which are found in recipient sera, may be released into the circulation from the injured graft or may be shed or secreted by donor dendritic cells (17, 18). These soluble MHC molecules may provide antigens for indirect allorecognition. Recent evidence from our and other laboratories has documented that MHC peptides derived from one MHC molecule can be presented to T cells by another MHC molecule (11–14, 22, 23). Both self- and allo-peptides bind to MHC class II molecules and elicit oligoclonal T cell proliferation (13, 21). Specific immunosuppressive therapy should, therefore, involve blockade of TCRs and/or of MHC binding sites.

The demonstration of T cell reactivity against exogenous MHC peptides bound to an MHC molecule, in our previous studies, was based on experiments in which T cells were sensitized to HLA-DR1 in vitro using autologous APC and synthetic DR1 peptides (13, 20, 21). The resulting TCC reacted to allogeneic DR1-positive cells only in the presence of autologous APC. When the DR1 molecule was coexpressed on the membrane of the same cell with the responder's HLA-DR-restrictive element (DRβ1*1101), the TCC was specifically stimulated, indicating that it recognized processed DR1 presented by the DR11 molecule. Residue 21–42 of the DRβ1*0101 chain was shown to comprise the T cell determinant region eliciting T cell reactivity against the stimulating DR1-positive cells.

Although the use of a panel of synthetic peptides for in vitro immunization permits the identification of T cell determinant regions, this approach leaves open the criticism that the sensitizing peptide may not be produced during the natural processing of the respective antigen by APC (24). Since indirect recognition is expected to evolve from direct recognition, which causes the release of alloantigen from injured donor cells and its processing and presentation by host APC, it was important to determine whether indeed the two events occur together during allostimulation.

We approached this problem in an in vitro system by sensitizing the responder's T cells in MLC with allogeneic DR1-positive cells. The MLC-primed T cells were then tested for direct recognition ability, i.e., for non-MHC-restricted reactivity to allogeneic DR1 cells and for indirect recognition, i.e., for MHC-restricted recognition of synthetic DR1 peptides. We found that the frequency of cells recognizing directly the alloantigen was ~100-fold higher than that of cells recognizing a DR1 peptide presented by autologous APC. The dominant epitope of DR1 recognized by the responding T cells in context of DRβ1*1201 lies within residue 21–42.

Of particular interest was the finding that TCC specific for this DR1 peptide were also triggered to proliferate strongly by cells expressing certain polymorphic variants of DR4 (DRβ1*0401, 0403, and 0404). Other variants of DR4, such as DRβ1*0402, failed to induce proliferation. This result is reminiscent of our previous finding that cells heterozygous for HLA-DR3 and -2 stimulate the reactivity of a TCC specific for the DR1 peptide 21–42 presented in context of the responder's DRβ1*0101 allele (21). In both of these cases there seems to be molecular mimicry between complexes formed by an HLA-DR molecule, such as DR1 or DR12 in the present study, with DR1 peptide 21–42 on one hand, and complexes involving an unrelated allogeneic HLA-DR molecule with its bound peptide on the other hand. In an attempt to explain this crossreactivity we examined the published amino acid sequence of DR4 allelic variants (25). The location of amino acids in the first and second allelic hypervariable region, corresponding to the floor of the antigen binding groove, is identical in all DRβ1*04 alleles with the exception of DRβ1*0406, which has serine instead of tyrosine in position 37. The major difference between the various DRβ1*04 alleles occurs in the third hypervariable region, which contains the T cell contact residues. These differences, however, do not permit grouping of the DR4 antigens in stimulatory and nonstimulatory categories, corresponding to the T cell reactivity pattern observed in the present study.

Since T cells capable of direct recognition recognize a binary complex of foreign MHC and a bound peptide, the crossreactivity of DR1 peptide 21–42 presented by DR12 and DRβ1*04 alleles is caused most likely by the conformation of this complex.

Our observation that molecular mimicry occurs when MHC peptides bind as processed fragments to an HLA-DR antigen for recognition by T cells has important clinical implication. First, it is possible that such complexes trigger autoimmune reactions, as has been previously suggested (26). Second, it is possible that sensitization to one alloantigen recognized by T cells in a primary graft leads to second set rejection of a subsequent graft carrying a different HLA phenotype. This hypothesis may explain at least in part the significantly lower survival of secondary grafts compared with primary grafts.

The contribution of the indirect pathway of allorecognition to alloimmunity has been documented in animal models (11, 12). Benichou et al. (12) showed that after immunization of mice with allogeneic spleen cells or skin grafts, the in vivo primed T cells proliferate in vitro in response to peptides corresponding to polymorphic regions of the allogeneic MHC class II molecule. Similarly, Fangmann et al. (11) demonstrated that rats immunized with allopeptides showed accelerated rejection of skin allografts carrying the MHC molecule whose sequence was used for allopeptide synthesis. Our data represent the first demonstration in humans that in vitro immunization with native HLA-DR molecule, expressed on the surface of allogeneic cells, leads to the generation of T cells that react with processed forms of the alloantigen. The frequency of such T cells increases from ~1:250,000 in un-

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primed population (13) to 1:40,000 after stimulation with allogeneic cells expressing the DR1 molecule. This reinforces the view that indirect recognition can play an important role in allograft rejection.

Finally, consistent with our previous finding of a limited and biased TCR-Vβ gene usage in allopeptide-specific T cells, in the present study we found that the DR1 peptide–specific TCL and the TCC derived from a DR12-positive responder exclusively used Vβ3. This finding supports the concept that TCR-targetted immunosuppressive therapy may be useful for suppression of indirect T cell alloreactivity and consequently of chronic allograft rejection.

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