Surface Expression of Functional T Cell Receptor Chains Formed by Interlocus Recombination on Human T Lymphocytes

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Summary

Structural diversity of lymphocyte antigen receptors (the immunoglobulin [Ig] of B cells and the α/β or γ/δ T cell receptor [TCR] of T cells) is generated through somatic rearrangements of V, D, and J gene segments. Classically, these recombination events involve gene segments from the same Ig or TCR locus. However, occurrence of "*trans*" rearrangements between distinct loci has also been described, although in no instances was the surface expression of the corresponding protein under normal physiological conditions demonstrated. Here we show that hybrid TCR genes generated by *trans* rearrangement between Vγ and (D) Jβ elements are translated into functional antigen receptor chains, paired with TCR α chains. Like classical α/β T cells, cells expressing these hybrid TCR chains express either CD4 or CD8 coreceptors and are frequently alloreactive. These results have several implications in terms of T cell repertoire selection and relationships between TCR structure and specificity. First, they suggest that TCR alloreactivity is determined by the repertoire selection processes operating during lymphocyte development rather than by structural features specific to VαVβ regions. Second, they suggest the existence of close structural relationships between γ/δ and α/β TCR and more particularly, between Vγ and Vβ regions. Finally, since a significant fraction of PBL (at least $1/10^4$) expressed hybrid TCR chains on their surface, these observations indicate that *trans* rearrangements significantly contribute to the combinatorial diversification of the peripheral immune repertoire.

Lymphocyte antigen receptor diversity results from somatic DNA recombination between genetic elements originally separated on the chromosome, the V, D, and J segments. This recombination is mediated by specific enzymes, the lymphoid recombinases, that catalyze the joining of elements flanked by recombination signal sequences (RSS)[1] comprising highly conserved heptamer and nonamer motifs (1, 2).

Ig and TCR gene rearrangements are activated in lymphoid cells only, at a specific developmental stage and in a sublineage-dependent fashion, i.e., complete Ig rearrangements occur in B cells only, whereas complete TCR rearrangements occur in T cells only. Several observations suggest that specificity of the recombination process depends on two main parameters: first, on the ability of a given cell to activate its recombination machinery, as this property is specific to developing lymphocytes, and second, on the ability of a cell to render its TCR or Ig loci accessible to recombinases, as this accessibility is tightly regulated within a given lymphoid sublineage (for a review see reference 3).

Classically, the IgH and L chains on B cells and the TCR α and β or γ and δ chains on T cells are encoded by genes formed by elements belonging to the same locus. However, because rearrangements between elements belonging to any Ig or TCR locus seem to be mediated by the same recombination complex (4, 5), "*trans*" rearrangements between elements belonging to distinct loci could in theory occur provided that both loci are accessible to the recombinases at the same developmental stage. Hybrid IgH/TCR α chain genes have been described in leukemic cells by several groups about a decade ago (6–9). More recently, the occurrence of trans rearrangements between TCR γ and β elements has been demonstrated not only in leukemic cells but also in normal PBL (10–14). However, in the absence of any evidence for surface expression of the corresponding protein on normal lymphocytes, the physiological significance of these interlocus rearrangements has thus far remained unclear. Here we show that

[1] *Abbreviations used in this paper:* BLCL, B lymphoblastoid cell line; DP, CD4/CD8 double positive; RSS, recombination signal sequence.

hybrid TCR genes comprising VDJ elements from TCR γ and β loci are translated into functional alloreactive TCR. These findings are discussed in terms of lymphoid lineage commitment, repertoire diversification, and TCR structure/specificity relationships.

Materials and Methods

Reagents and Cells. The following mAbs were used for flow cytometry analyses and immunomagnetic separations: 510 (pan δ [15]), 360 (anti-Vγ9 [15]), 23D12 (anti-Vγ2, 3, 4 [16]), 4A11 (anti-Vγ4 [17]), BMA031 (pan β [18]), TCRδ1 (pan δ [19]), CγM1 (pan γ [20]), WT31 (anti-CD3ϵ [21]), and F1 (anti-Vα2 [22]). F1 mAb was purchased from T-cell Diagnostics (Cambridge, MA). All the other mAbs were kind gifts from Drs. F. Romagné (Immunotech, Marseilles, France), M. Brenner (Harvard Medical School, Boston, MA), W. Tax (National Cancer Institute, Amsterdam, The Netherlands), A. Moretta (Instituto per la Ricerca sul Cancro, Genoa, Italy), and G. De Libero (Kantonspital, Basel, Switzerland).

The following B lymphoblastoid cells (BLCL) and mAbs used for functional assays were obtained from the VIIth HLA Workshop (New York, 1987): antibodies: D1.12 (anti-DR), Leu10 (anti-DQ), PL15 (anti-DP), and W6/32 (anti-HLA class I framework); BLCLs: RM (RML), IB (IBW9), BT (BTB), BO (BOLETH), SP (SP0010), and TU (TUBO).

Generation of γ/δ T Cell Clones and Lines. PBL from healthy donors were sorted, cultured, and cloned as previously described (23 and 24). In brief, cells were incubated with TCR V or C region-specific mAb for 45 min, washed once, and rotated for 4 h at 4°C with magnetic beads coated with sheep anti-mouse Ig (Dynal, Oslo, Norway). After eight washes, bead-adherent cells were cultured in medium (RPMI 1640, 10% human serum, 1 mM L-glutamine, rIL-2, leukoagglutinin [0.5 μg/ml]), and irradiated PBL and BLCL. Beads were removed at day 5, cells were cultured further, and at day 14, they were subjected to a second immunomagnetic separation. After two more weeks of culture, cells were cloned by limiting dilution in culture medium and irradiated feeder cells. Growing colonies with a probability of monoclonality >95% were kept for further analysis.

Flow Cytometry Analysis and Comodulation Assays. Cells were phenotyped by indirect immunofluorescence as previously described (24). In brief, cells were incubated first with unconjugated V- or C-specific mAb for 30 min on ice, washed, and incubated with FITC-conjugated rabbit anti-mouse Ig antiserum for 30 min on ice. For single-color immunofluorescence, cells were resuspended in PBS after one washing, and analyzed by flow cytometry. For two-color immunofluorescence, cells were incubated for 10 min at room temperature with normal mouse serum, washed, then incubated for 30 min on ice with biotinylated mAb, washed, and finally, incubated for 30 min on ice with PE-conjugated streptavidin (Immunotech, Marseilles, France). After washings, cells were analyzed by flow cytometry on a FACScan® apparatus (Becton Dickinson & Co., Mountain View, CA) using the LYSYS II software.

Comodulation assays were performed as follows: cells were incubated with irrelevant or biotinylated 23D12 mAb for 30 min on ice, washed twice, and then incubated overnight in avidinated microwell plates (Immunotech; 10^5 cells per well). After two washes, cells were stained and analyzed by flow cytometry as described above.

Functional Assays. Proliferation and cytotoxicity assays were performed as previously described (24, 25). In brief, cytotoxic activity of T cell clones was measured by a standard 4-h ^{51}Cr-release assay at 9:1 and 3:1 E/T cell ratios. Percent specific lysis was calculated as described previously (25). Proliferative activity of responder cells was estimated after a 48-h culture with irradiated BLCL followed by an overnight pulse with tritiated thymidine.

Amplification and Sequencing of TCR Transcripts. Preparation of T cell clone RNA, reverse transcription, amplification, and sequencing of VγCβ transcripts were performed as previously described (23) using VγI (16) and Cβ (5′ GGG AGA TCT CTG CTT CTG ATG GCT C) primers. TCR α transcripts were analyzed by anchored PCR as described earlier (26) using Cα (5′ GTT AGG ATC CTG TTT CAA AGC TTT TCT CGA CCA GC for reverse transcription; 5′ CTT TGT GAC ACA TTT GTT TGA G for second step amplification and sequencing) and polyT (26) primers.

Results and Discussion

Characterization of a PBL Subset Recognized by both Vγ- and Cβ-specific mAb. In the course of the characterization of a mAb termed 23D12, which turned out to be directed against a determinant shared by Vγ2, Vγ3, and Vγ4 TCR variable regions (16), several 23D12$^+$ polyclonal cell lines were generated after immunomagnetic sorting of PBL from healthy donors. Whereas the great majority of 23D12$^+$ lymphocytes were recognized by the pan δ mAb #510 (15) and thus, presumably expressed a γ/δ TCR, a minor subset recognized

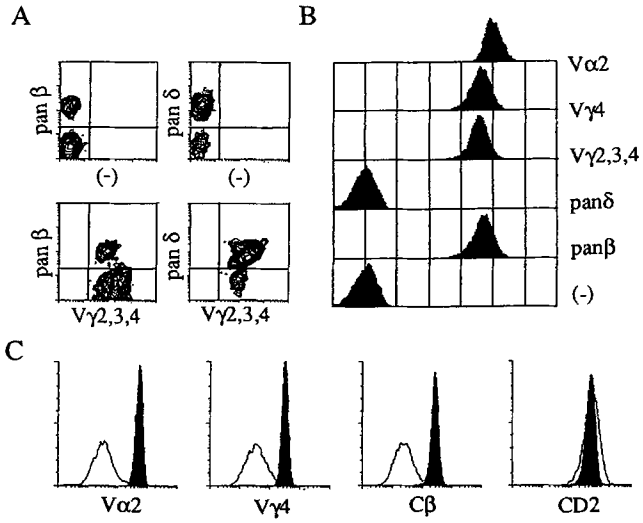

Figure 1. (*A*) Two-color flow cytometry analysis of sorted 23D12$^+$ T cells using pan β, pan δ, and Vγ2, 3, 4-specific mAb. Shown are the fluorescence contour plots (log scale) of cells from line #83 (Table 1), stained by immunofluorescence using PE-conjugated BMA031 (*panβ*) or 510 (*panδ*) mAb (*ordinate*, red fluorescence), and purified 23D12 (Vγ2,3,4-specific) mAb, whose binding was revealed by FITC-conjugated rabbit anti-mouse Ig (*abscissa*, green fluorescence). (*B*) Single-color flow cytometry analysis of a 23D12$^+$BMA031$^+$ T cell clone. Vα2$^+$BMA031$^+$23D12$^+$ T cells were isolated after sequential immunomagnetic sorting of PBL using 23D12, BMA031, and a Vα2.1-specific mAb (T-cell Sciences). Shown are the fluorescence histograms (log green fluorescence) using mAb whose specificity is shown in the figure. (*C*) Comodulation of Cβ, Vα2, and Vγ4 epitopes on a 23D12$^+$BMA031$^+$ T cell clone. Cells were incubated overnight with (*white histograms*) or without (*black histograms*) cross-linked 23D12 mAb, washed, and stained by indirect immunofluorescence using CD2, Cβ-, Vα2-, and Vγ4-specific mAbs. Note the partial modulation of the Cβ, Vα2, and Vγ4 epitopes, but not of CD2 epitopes, after overnight incubation with 23D12 mAb.

by the pan β mAb #BMA031 (18) but not by the pan δ mAb was reproducibly detected in most lines analyzed (Fig. 1 *A* and Table 1). The proportions of BMA031$^+$ cells among 23D12$^+$ T cells were generally 1%, and in about 1/4 of the cell lines studied, were >10% (Table 1 and data not shown). This indicated that cells with this peculiar phenotype were rather frequent under normal physiological conditions ($\geq 1/10^4$ PBL considering that 23D12$^+$ cells represent on average 1% of PBL [16]). To rule out a possible mAb cross-reactivity problem, additional staining experiments were performed on 23D12$^+$BMA031$^+$ T cell clones using a large panel of TCR V and C region-specific mAbs. Parts of 23D12$^+$BMA031$^+$ cells were recognized by a Vγ4-specific mAb (4A11 [17]) (Fig. 1 *B* and Table 1). Moreover, all of them were stained by the Cβ-specific mAb βF1 (20), as well as by the WT31 mAb (21), which is directed against a CD3ϵ epitope exposed on α/β but not on γ/δ T cells (data not shown). In contrast, 23D12$^+$BMA031$^+$ cells were recognized neither by the Cγ-specific mAb CγM1 (20), nor by the Cδ-specific mAb TCRδ1 (19) (data not shown). Taken together, these results confirmed the presence of Vγ and Cβ epitopes and rule out expression of TCR γ and δ chains on 23D12$^+$BMA031$^+$ clones.

All 23D12$^+$BMA031$^+$ Clones Express Productively Rearranged TCR Genes Comprising Vγ and Cβ Elements. In light of recent studies demonstrating the occurrence of TCR chain gene rearrangements between TCR γ and β loci (10, 11), we suspected the presence of a hybrid TCR chain comprising Vγ and Cβ regions on the surface of 23D12$^+$BMA031$^+$ T cells. To test this directly, we performed PCR amplifications on mRNA derived from several 23D12$^+$BMA031$^+$ clones using primers specific to Cβ and to a DNA region shared by Vγ2, Vγ3, and Vγ4 elements. A DNA band of the expected size was amplified in all the clones tested using this pair of primers. Moreover, sequencing of the amplified cDNA demonstrated the occurrence of a productive *trans* rearrangement between Vγ and Jβ elements in all but one case (Fig. 2). Some but not all transcripts comprised a Dβ element, suggesting that γ/β *trans* rearrangements could occur both before or after partial DJβ rearrangements. An unusual hybrid sequence formed by a recombined VγJγ exon spliced to a Cβ exon was found in one clone (#71.13, Fig. 2). It has been previously proposed that this kind of transcript could result from a *trans* splicing between mRNA from rearranged TCR γ locus and unrearranged TCR β locus (11). The fact that such a structure was stably expressed in a T cell clone probably rules out a *trans* splicing event but rather suggests the occurrence of a secondary interlocus recombination following or preceding a normal intralocus VγJγ rearrangement. It is not clear at that stage whether this recombination event involves classical heptamer/nonamer RSS flanking the TCR V, D, and J elements or yet another signal sequence. In sup-

Table 1. *Frequency, Vγ Usage, and CD4/CD8 Coreceptor Expression by 23D12$^+$BMA031$^+$ T Cells Estimated by Flow Cytometry*

PBL line	TCR-β among 23D12$^+$	TCR-δ among 23D12$^+$	Vγ4 among 23D12$^+\alpha/\beta^+$	CD4 among 23D12$^+\alpha/\beta^+$	CD8* among 23D12$^+\alpha/\beta^+$	Vγ4 among 23D12$^+\gamma/\delta^+$	CD4 among 23D12$^+\gamma/\delta^+$	CD8* among 23D12$^+\gamma/\delta^+$
67	1.3	98.8	99.8	0.6	99.9	20.2	0.6	0.0
69	6.0	94.1	87.1	98.3	4.5	20.0	0.0	1.6
70	3.0	97.3	57.0	19.4	80.3	47.3	2.8	.0
71	1.9	98.2	47.4	18.8	53.2	29.1	0.5	1.0
72	0.0	99.6	(-)‡	(-)	(-)	26.5	0.5	1.0
73	17.0	83.7	0.2	0.7	89.5	1.2	2.0	5.0
76	1.6	98.3	0.6	17.0	82.0	29.5	0.7	0.0
83	13.4	85.6	88.4	26.5	37.0	40.0	6.6	0.0
36	25.4	74.8	98.5	0.8	92.6	95.7	ND	ND
37	3.6	96.1	97.6	5.1	57.3	89.4	ND	ND
38	5.0	96.0	99.9	3.9	96.0	34.9	ND	ND
39	2.0	98.3	97.0	13.2	82.9	74.4	ND	ND
Mean	6.7	93.4	70.3	18.6	70.4	42.6	1.8	1.2

23D12$^+$ cultured PBL were recognized in a mutually exclusive fashion by the pan β (BMA031 [18]) and the pan δ (510 [15]) mAb. The percentage of Vγ4$^+$ (4A11$^+$ [17]) cells was significantly higher among 23D12$^+\alpha/\beta^+$ than among 23D12$^+\gamma/\delta^+$ lymphocytes (Wilcoxon matched pairs test, $p = 0.02$). Note that unlike 23D12$^+\gamma/\delta^+$ cells, most 23D12$^+\alpha/\beta^+$ cells expressed either CD4 or CD8 coreceptors.

* Percentage of CD8 bright.

‡ No 23D12$^+$BMA031$^+$ line could be derived from this individual. The percentage of Vγ4$^+$, CD4$^+$ and CD8$^+$ cells among 23D12$^+\alpha/\beta^+$ was estimated on sorted 23D12$^+$BMA031$^+$ PBL. The percentage of Vγ4$^+$, CD4$^+$, and CD8$^+$ cells among 23D12$^+\gamma/\delta^+$ lymphocytes was deduced from a combined analysis of 23D12$^+$ and 23D12$^+\alpha/\beta^+$ T cells.

Clone	Rearrangment	Vγ	NDN	Jβ	Rearrangment	Vα	N*	Jα
C36.2	Vγ4Dβ2.1Jβ2.1	CATWD	TGTSGRASSH	NEQFF	Vα13.1Jα42	CA	VEGY	GGSQGNLIF
C36.6	Vγ4 Jβ2.2	CATWD	A	NTGELFF	Vα2.4 Jα30	CAM	SGX	RDKIIF
C36.17	Vγ4 Jβ2.3	CATW	RI	TDTQYF	Vα13.1Jα22	CA	VAVS	SSGSAKRQLTF
C37.38	Vγ4 Jβ1.2	CATWDG	LAT	GYTF	Vα3.1 Jα29	CAT	XI	GNTPLVF
C37.40	Vγ4 Jβ1.6	CATW	EGI	NSPLHF	Vα27.1Jα44	CA	VVGVL	TASKLTF
C37.47	Vγ4		-		VαX Jα45	CAA	SYDGN	SGGGAGDLTF
C36.1	Vγ4 Jβ1.3	CAT	PTDR	NTIYF			-	
Cco	Vγ4Dβ1.1Jβ2.1	CAT	PGGVY	NEQFF			-	
C71.13	Vγ4 JγP2	CATWDG	GGG	DWIKTF	Vα28.1Jα34	CA	VKG	NTDKLIF
C76.1	Vγ3Dβ1.1Jβ1.6	CAT	SWGPIL	SPLHF	Vα4.2 Jα36	CI	VRVLA	GANNLF
C76.3	Vγ3Dβ2.1Jβ2.5	CATWD	LGTLTE	ETQYF	Vα7.2 Jα10	CA	VRHVV	TGGGNKLTF
C76.16	Vγ3 Jβ2.3	CATW	G	TDTQYF	Vα30 Jα52	CA	VQAK	GGTSYGKLTF
C70.1	Vγ3 Jβ2.3	CATWD		TDTQYF	Vα23.1Jα54	CA	VT	IQGAQKLVF

Figure 2. Deduced amino acid sequences of TCR transcripts expressed in 23D12$^+$BMA031$^+$ T cell clones. Clones were derived from six individuals (#36, 37, co, 71, 76, and 70). All clones were stained by 23D12 and BMA031 mAbs (Vγ2,3,4-, and TCR-β-specific, respectively). All clones except C76.1, C76.3, C76.16, and C70.1 were also recognized by the Vγ4-specific mAb 4A11. V(D)J assignments were deduced from a comparative analysis of cDNA sequences derived from 23D12$^+$BMA031$^+$ T cell clones with those of known VDJ elements. The following TCR nomenclatures are used throughout the manuscript: Vα (from the XIth International HLA Workshop, 1993, Japan), Jα (37), Jβ (38), Vγ (39), and Jγ (39). The Vα gene expressed by clone C37.47 (termed *VαX*) is closely related to AF211 (40). Clone C71.13 expressed a TCR β chain gene comprising Vγ4JγP2 exon, normally spliced to a Cβ2 exon (data not shown). (*NDN*) Nongermline and Dβ-encoded amino acids. In the case of TCR α, most available Vα gene sequences were derived from cDNA clones. Therefore the assignment of a given junctional nucleotide or amino acid to Vα or to the N region could not be ascertained. (-) Not done. These sequence data are available from EMBL/GenBank/DDBJ under accession numbers X81536–X81556.

port of the second possibility, the three examples of VγJγCβ rearrangements described here and elsewhere (11) involved the same Jγ segment (JγP2), which would suggest the existence of a recombination hot spot located downstream of JγP2, and presumably upstream of Jγ2. These results raise the possibility that all hybrid TCR γ/β rearrangements are preceded by a common translocation event. In support of this, a preliminary Southern blot analysis of DNA from several T cell clones carrying either VγJγCβ, VγDβJβCβ, or VγJβCβ rearrangements demonstrated the existence of a common nonassignable band hybridizing with the Jγ probe pH60 (27 and our unpublished observations). An in-depth molecular analysis of several of these clones is currently underway. Taken together, these results suggest the existence of a primary recombination event possibly involving signal sequences distinct from the Ig and TCR RSS. If so, understanding its precise mechanism might provide clues on the etiology of diseases such as ataxia telangiectasia, where the frequency of *trans* rearrangements between Vγ and Cβ is on average 10–50-fold higher than in normal individuals (11).

Hybrid TCR VγCβ Chains Are Paired to Classical VαCα Chains. In several polyclonal lines, a significant fraction of 23D12$^+$BMA031$^+$ T cells was recognized by a Vα2-specific mAb (data not shown). Moreover, productive TCR α chain gene transcripts were detected in all the 23D12$^+$BMA031$^+$ clones studied (Fig. 2), suggesting that the hybrid TCR β chain was paired with a TCR α chain. To formally prove this, we isolated a Vα2$^+$23D12$^+$BMA031$^+$ T cell clone after immunomagnetic sorting (Fig. 1 *B*), confirmed the presence of Vγ4Cβ and Vα2Cα transcripts, and then, demonstrated a physical association between the Vγ4Cβ and the V2JCα TCR chains by comodulation experiments using Cβ-, Vγ-, and Vα-specific mAbs (Fig. 1 *C*).

Evidence for a Preferential Usage of Particular Vγ but not Vα Region Genes by VγCβ$^+$ Cells. A previous analysis of hybrid VγCβ gene rearrangements derived from normal PBL suggested a biased Vγ gene usage in favor of Vγ4 (11). Accordingly, the proportions of cells recognized by the Vγ4-specific mAb 4A11 (17) were significantly higher among VγCβ$^+$ than among VγCγ$^+$ cells (Table 1). Moreover, whereas we could derive cells expressing hybrid VγCβ chains using either Vγ2, Vγ3, or Vγ4 genes from 19/20 PBL samples (Table 1 and data not shown), we were unable to isolate cells expressing Vγ9Cβ chains despite numerous attempts. In contrast to Vγ, the Vα repertoire of 23D12$^+$α/β$^+$ T cells was not biased toward usage of a particular Vα region. Indeed, analysis of TCR Vα gene expression in polyclonal 23D12$^+$α/β$^+$ T cell lines using a panel of primers specific to the 29 Vα families demonstrated usage of distinct sets of Vα genes from one line to another (data not shown). Accordingly, productive Vα transcripts derived from the 23D12$^+$α/β$^+$ T cell clones studied comprised diverse V region genes (Fig. 2).

The process leading to underrepresentation of Vγ9Cβ$^+$ and overrepresentation of Vγ4Cβ$^+$ cells irrespective of their Vα region is yet unclear. During development, rearrangements involving C-proximal V genes, such as Vγ9, are activated at earlier stages than those involving C-distal V genes, such as Vγ2, 3, and 4 (28). Perhaps Vγ9 rearrangements occur at a stage when the whole β locus is not yet accessible to recombinases and thus, accessibility or combinatorial constraints might account for the absence of Vγ9Cβ$^+$ cells. However, this cannot explain a biased Vγ gene usage in favor of Vγ4 because such a bias was observed among productive but not among nonproductive hybrid rearrangements involving Vγ elements from the first Vγ family (11). It is possible that the structure of some Vγ regions is more or less compatible with stable conformation of the hybrid VγCβ chain and/or proper pairing with TCR α chains. Also, biased usage of Vγ but not Vα region by VγCβ$^+$ cells might result from an in vivo selection by superantigens, whose recognition classically imposes constraints on one of the TCR V regions only (29). In this respect, some Vγ regions, like most Vβ regions, show affinity for certain staphylococcal superantigens (30).

CD4 and CD8 Coreceptor Expression by VγCβ$^+$ Cells. During their intrathymic development, α/β and γ/δ T cells follow two distinct differentiation pathways. Whereas most γ/δ thymocytes remain CD4$^-$CD8$^-$ throughout development, almost all α/β T cells go through a CD4$^+$

$CD8^+$ (double positive [DP]) stage (for a review see reference 31). Positive selection of these immature DP α/β thymocytes by self-peptide–MHC complexes leads then to their terminal differentiation into $CD8^+CD4^-$ or $CD8^-CD4^+$ mature thymocytes (31). Because almost all $23D12^+\alpha/\beta$ T cells expressed either CD4 or CD8 coreceptors, unlike their γ/δ counterparts (Table 1), they probably differentiated from DP cells. Recent studies (32) have shown that transition to the DP stage tightly depends on the occurrence of a productive TCR β rearrangement. Therefore, the fact that CD4 and CD8 expression was induced after either $V\beta DBJ\beta C\beta$ or $V\gamma(D\beta)$ $J\beta C\beta$ rearrangement within the precursor cell would indicate that transition to the DP stage is primarily dictated by the locus origin of the constant rather than the variable part of the rearranged gene, which is in accordance with recent observations made by others (33).

Alloreactivity of $V\gamma C\beta^+$ Lymphocytes. Unlike γ/δ T cells, a large fraction of α/β T cells is reactive against polymorphic allogeneic MHC molecules. This allorecognition probably reflects the close structural relationships between the allogeneic MHC molecules recognized by mature α/β T cells and the self-MHC–peptide complexes against which immature α/β T cells have been positively selected (34). Analysis of the reactivity of polyclonal $23D12^+\alpha/\beta^+$ T cell lines toward six randomly chosen allogeneic B cells revealed strong proliferative (Fig. 3 *A*) or cytotoxic (Fig. 3 *D*) responses of most lines against one or more allogeneic cells. Moreover, alloresponses of some $23D12^+\alpha/\beta^+$ clones derived from these lines (Fig. 3 *B*) were blocked by the W6/32 mAb, which is directed against a constant part of classical MHC class I molecules (Fig. 3, *C* and *D*). Together with the presence of either CD4 or CD8 coreceptors on most $23D12^+\alpha/\beta^+$ T cell clones (Table 1), these results strongly suggest that the ligands recognized by $23D12^+\alpha/\beta^+$ cells are very similar to those recognized by "classical" α/β T cells.

These observations have several implications with respect to TCR selection processes and structural relationships between α/β and γ/δ TCR. Since most γ/δ cells (including $23D12^+$ cells) are $CD4^-CD8^-$ and are not alloreactive (35), this would indicate that the same V region can participate in the recognition of distinct sets of ligands depending on the TCR chain to which it is associated. These results also suggest a close structural relationship between $V\gamma$ and $V\beta$ regions, an assumption which is also supported by the fact that both V regions display superantigenic reactivity (29, 30), unlike $V\alpha$ and $V\delta$ regions. A nice parallel might be drawn between the relative interchangeability between $V\beta$ and $V\gamma$ regions, which is supported by the present data, and the $V\alpha/V\delta$ interchangeability, which is suggested by studies demonstrating the occurrence of $V\delta C\alpha$ rearrangements (36). It should be mentioned, however, that given the peculiar organization of the α/δ locus, $V\delta C\alpha$ chains, unlike $V\gamma C\beta$ chains, are formed by a classical intralocus rearrangement. In light of these observations, one could imagine the very same $V\gamma V\delta$ combination to be associated with either $C\alpha C\beta$ or $C\gamma C\delta$ regions. By comparing the antigenic specificity of these cells, it should be possible to directly test whether alloreactivity is a feature acquired through intrathymic TCR selection or whether it is dictated by germline motifs on $V\alpha$ and/or $V\beta$ regions.

Concluding Remarks. Here we have shown that a significant fraction of peripheral α/β T cells expressed surface hybrid receptors formed by interlocus recombination between TCR $V\gamma$ and (D) $J\beta$ elements. Moreover, these cells displayed most features of mature α/β T cells and expressed functional alloreactive TCR. Hence, these results indicate that TCR *trans* rearrangements represent a novel mode of diversification that truly contributes to the combinatorial diversity of the peripheral immune repertoire.

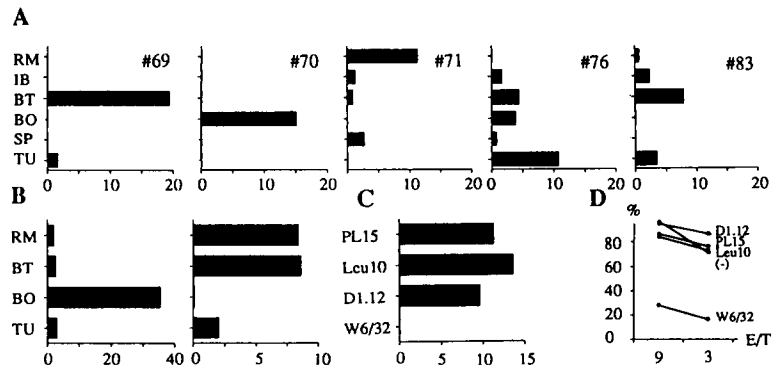

Figure 3. Functional analysis of alloresponses mediated by $23D12^+\alpha/\beta^+$ T cells. (*A*) Proliferative response of polyclonal $23D12^+\alpha/\beta^+$ T cell lines after coculture with allogeneic BLCL. Polyclonal $23D12^+BMA031^+$ lines (>99% pure) were cultured for 48 h in the presence of randomly chosen irradiated allogeneic BLCL, and pulsed for 18 h with tritiated thymidine ([^{3}H]TdR). Shown are the proliferative responses ([^{3}H]TdR uptake expressed in cpm $\times 10^{-3}$) of five cell lines (*#69*, *70*, *71*, *76*, and *83*) against six BLCL (*RM*, *IB*, *BT*, *BO*, *SP*, and *TU*). (*B*) Proliferative alloresponses of two $23D12^+\alpha/\beta^+$ T cell clones. Shown are the proliferative responses (expressed in cpm $\times 10^{-3}$) of two $23D12^+BMA031^+$ T cell clones derived from line #76 (clone #76.3: $CD8^+$ [*left*] and #76.1: $CD4^+$ [*right*]; see Fig. 2) against four allogeneic BLCL (*TU*, *BO*, *BT*, and *RM*). Several other alloreactive clones derived from this and other lines were obtained but are not shown here. (*C*) Blockage of allogeneic BLCL-induced proliferation of a $23D12^+\alpha/\beta^+$ T cell clone by anti-HLA mAb. The proliferative activity of a $23D12^+BMA031^+$ T cell clone derived from line #76 (#76.3: $CD8^+$, see Fig. 2) was estimated in the absence or presence of mAb directed against HLA framework determinants: W6/32 (anti-HLA I), D1.12 (anti-HLA DR), Leu10 (anti-HLA DQ), and PL15 (anti-HLA DP). Results are expressed in cpm $\times 10^{-3}$. (*D*) Blockage of $23D12^+\alpha/\beta^+$ T cell–mediated cytolysis of an allogeneic BLCL by anti-HLA mAb. Cytolytic activity of $23D12^+BMA031^+$ cells (line #70) against the allogeneic BLCL BO was estimated in the absence (−) or presence of anti-HLA framework mAb (see *C*). Results are expressed as percent specific target lysis (*ordinate*) calculated at 9:1 and 3:1 E/T ratios. Similar results were obtained with the $23D12^+\alpha/\beta^+$ T cell clone #70.1 derived from this line (Fig. 2 and data not shown).

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