Brief Definitive Report

An Invariant Vα24–JαQ/Vβ11 T Cell Receptor Is Expressed in All Individuals by Clonally Expanded $CD4^-8^-$ T Cells

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Summary

The T cell receptor (TCR)-α/β $CD4^-8^-$ (double negative, DN) T cell subset is characterized by an oligoclonal repertoire and a restricted V gene usage. By immunizing mice with a DN T cell clone we generated two monoclonal antibodies (mAbs) against Vα24 and Vβ11, which have been reported to be preferentially expressed in DN T cells. Using these antibodies, we could investigate the expression and pairing of these Vα and Vβ gene products among different T cell subsets. Vα24 is rarely expressed among $CD4^+$ and especially $CD8^+$ T cells. In these cases it is rearranged to different Jα segments, carries N nucleotides, and pairs with different Vβ. Remarkably, Vα24 is frequently expressed among DN T cells and is always present as an invariant rearrangement with JαQ, without N region diversity. This invariant Vα24 chain is always paired to Vβ11. This unique Vα24–JαQ/Vβ11 TCR was found in expanded DN clones from all the individuals tested. These findings suggest that the frequent occurrence of cells carrying this invariant TCR is due to peripheral expansion of rare clones after recognition of a nonpolymorphic ligand.

Growing evidence indicates that TCR-α/β $CD4^-8^-$ (double negative, DN) T cells constitute a peculiar subset with respect to ontogeny, specificity, lifespan, and TCR V gene usage. These cells do not appear to undergo classical positive and negative selection in the thymus (1–4), and it has been suggested that they may develop extrathymically (5–7). With respect to specificity, it has been shown that in mice α/β DN T cells recognize bacteria (8), and in humans, recognize monomorphic CD1 molecules as such or in association with bacterial products (9–11). A particularly striking feature is the presence within this population of expanded clones that persist for years with the same clonal size (11, 12). Furthermore α/β DN T cells display a restricted TCR V gene usage (13, 14). Molecular analysis of random cDNAs from human α/β DN T cells revealed a preferential expression of particular Vα and Vβ such as Vα24 and 7 and Vβ2, 8, 11, and 13 (13, 15) suggesting a biased TCR usage. However, this method could not address whether there could be a preferential pairing of the various V gene products and whether the frequent occurrence of a given sequence is due to a frequent rearrangement or to the clonal expansion of the corresponding T cells.

We have previously isolated from a healthy donor an expanded α/β DN T cell clone (CO9) that expresses Vα24 paired to Vβ11. The CO9 Vα sequence consists of a Vα24–JαQ rearrangement with no N region. This sequence was already reported in a leukemic patient (16), and can be detected by PCR-oligotyping in peripheral blood lymphocytes of all donors tested (11). Furthermore Porcelli et al. (13) reported that this sequence could be detected in cDNA isolated from DN T cells.

To identify the possible existence of this clonotype in different subsets and individuals we immunized mice with the CO9 clone and produced two monoclonal antibodies to Vα24 and Vβ11. Using these reagents we could show that expanded clones carrying the invariant Vα24–JαQ paired to Vβ11 TCR are present in all individuals in the α/β DN T cell subset. This remarkable TCR conservation and clonal expansion suggest a major role for nonpolymorphic highly conserved ligands in the selection of the α/β DN T cell repertoire.

Materials and Methods

mAbs and Flow Cytometry. Two mouse mAbs were produced from mice immunized with T cell clone CO9 (11). The anti-Vα24 mAb (C15, IgG1) recognizes Vα24 independently of Vβ and Jα and has been already described (17). From the same fusion we isolated an anti-Vβ11 antibody (C21, IgG2a). T cells stained by this antibody were sorted and cloned. All the clones analyzed express Vβ11 with different N regions, Jβ and Vα (this work and Dellabona, P., unpublished results). Anti-CD8 (OKT8, IgG2a) was obtained from American Type Culture Collection (Rockville, MD) and anti-CD4 (10A12, IgG2a) was a gift of Dr. Eddy Roosnek (Hôpital Cantonal Universitaire, Geneva, Switzerland). All the

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antibodies were used in indirect immunofluorescence followed by subclass-specific FITC- or PE-conjugated goat anti-mouse antibodies (Southern Biotechnology Associates, Birmingham, AL). The stained cells were analyzed on a FACScan® or sorted on a FACStar plus® (Becton Dickinson & Co., Mountain View, CA).

PCR Reaction and Sequencing. The methods for isolation and maintenance of T cell clones have been previously described (11). PCR reactions were carried out as described (11). Briefly, total RNA was extracted from T cell clones, reverse transcribed into cDNA, and amplified using the following oligonucleotides: Vα24 5′-GCAACTGTCGACGCAGACACAAAGCAGAGC-3′; JαQ 5′-GGATAGAATTCCAGACGGTCAACTGAGTTCC-3′; Vβ11 5′-GACCCTGAATTCTGCCAGGCCCTCACATACCTCTCA-3′; Jβ2.1 5′-GTTCAGGACGACTAGCACGGTGAGCCCTGTCCCTGG-3′; Cα 5′-TGCTCTTGAATTCCATAGACCTCATGTC-3′; and Cβ 5′-TGCTGACCCCACTGTCGACCTCCTTCCCATT-3′. PCR products were fractionated on low melt agarose gels, eluted, and directly sequenced (18) using the following primers: Cα 5′-CAGACAGACTTGTCACT-3′; and Cβ 5′-TGCTTCTGATGGCTCAA-3′.

Oligotyping and Heteroduplex Analysis. Oligotyping on PCR products was performed according to a published protocol (11), using the following ^{32}P-labeled oligonucleotides as probes: Cα 5′-CAGACAGACTTGTCAACT-3′; JαQ 5′-GGATAGAATTCCAGACGGTCAACTGAGTTCC-3′; invariant Vα24-JαQ junction (N-CO9) 5′-TGGTGAGCGACAGAG-3′.

For heteroduplex analysis (19) the PCR products were heated at 94°C for 5 min, cooled down at 64°C for 1 h and run on a 12% native acrylamide gel in 0.5× Tris borate EDTA buffer at 15 mA for 12 h at 4°C. The gels were stained with ethidium bromide and photographed under UV light.

Results and Discussion

Vα24 Is Expressed in Association with Vβ11 on α/β DN T Cells. We have previously described an expanded α/β DN clone (CO9) that expresses Vα24 and Vβ11 (11). Since these two V genes are frequently found among random cDNA clones generated from DN cells (13), we were interested to study whether this pairing would be selected in the DN subset of all individuals.

By immunizing mice with CO9 we obtained two mAbs recognizing Vα24 and Vβ11 (17 and our unpublished observations). By two-color staining, we found that cells expressing Vα24 are very rare (~0.1%) among CD8$^+$ T cells, but more frequent (~0.5%) in the CD4$^+$ and especially in the DN subset, where they may account for a substantial fraction (2–10%) of all cells (data not shown). To analyze the pairing of Vα24 with Vβ, we first sorted Vα24$^+$ T cells from the CD4$^+$, CD8$^+$, and DN T cell subsets. The sorted cells were expanded in short-term polyclonal lines and analyzed by two-color fluorescence for the expression of Vα24 and Vβ11. Strikingly, in 4 out of 5 individuals, all Vα24$^+$ cells within the DN compartment express Vβ11, whereas in one, about half of the cells express this Vα/Vβ pair (Fig. 1 and Table 1). In the CD4$^+$ compartment, T cells expressing Vα24 and Vβ11 are present as a minor fraction of the total Vα24$^+$ cells whereas they are absent in the CD8 compartment.

The Vα24 Paired to Vβ11 in DN T Cells Is Identical to the CO9 Chain. To dissect the structure of the Vα24 TCR chains expressed in DN, CD4$^+$, and CD8$^+$ T cells, we prepared

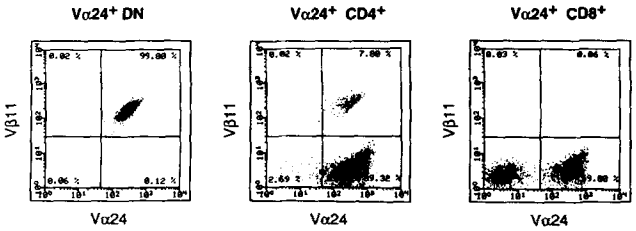


Figure 1. Vα24 pairs selectively with Vβ11 within the α/β DN T cell compartment. Vα24$^+$ T cells were sorted from the CD4$^+$, CD8$^+$, and DN compartment (donor ALA), expanded, and analyzed for expression of Vα24 and Vβ11 by two-color fluorescence.

cDNA from the short-term Vα24$^+$ polyclonal T cell lines. The cDNA was amplified with Vα24- and Cα-specific oligonucleotides to obtain a fragment encompassing the V-J junction. The PCR product was fractionated on agarose gels, blotted, and hybridized with oligonucleotide probes specific for Cα, JαQ, and the V-J junction of CO9 to detect the expression of total Vα24, JαQ, and of the CO9 Vα24–JαQ junction that lacks N region diversity. As shown in Fig. 2 *A*, in all three donors studied the CO9 invariant Vα24–JαQ junction was prominent among DN T cells, was expressed at a much lower level among CD4$^+$, and was absent in CD8$^+$ cells. A similar pattern of expression was observed for JαQ indicating that this Jα is used preferentially for this type of invariant α chain. Note, however, that CD4$^+$ clones with Vα24–JαQ rearrangement but N region addition can be found, for example clone T6, which is shown as a control for the oligotyping (Fig. 2 *A*).

To get additional information on the heterogeneity of the Vα24 chains in the different T cell subsets, the same Vα24–Cα PCR products were subjected to heteroduplex analysis. As shown in Fig. 2 *B*, PCR products from the DN cells are substantially homogeneous since they gave a prominent band of homoduplex. Direct sequencing of the homoduplex band from donors CDO and FOL gave the exact CO9 junctional sequence (data not shown and Table 2), indicating that the dominant Vα24 sequence present among the DN subset is identical to CO9. In contrast, the product amplified from CD4$^+$ or CD8$^+$ T cells gave a clear heteroduplex pattern indicating a substantial heterogeneity of the products, although in some cases clear bands of homo- and heteroduplexes were superimposed on the polyclonal pattern.

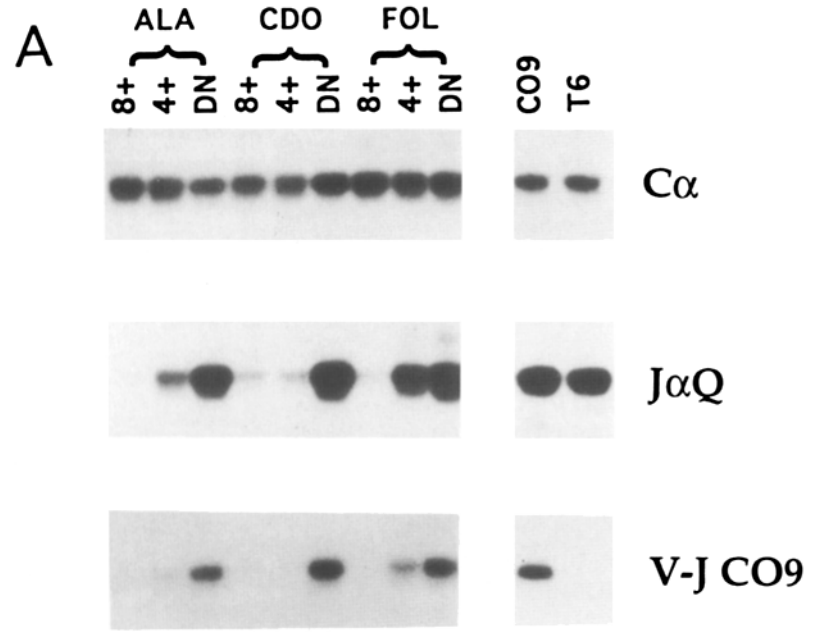
We conclude that the invariant Vα24–JαQ chain is found mainly among the DN cells of all individuals and, in some individuals, also in the CD4$^+$ compartment, although at a lower level.

TCR Structure of Vα24$^+$/Vβ11$^+$ DN T Cell Clones. The previous results show that DN T cells express an invariant Vα24 paired with Vβ11. To determine the structure of the associated β chain we directly sorted peripheral T cells stained by both anti-Vα24 and anti-Vβ11 antibodies and isolated a panel of 48 independent T cell clones. The cDNA prepared from each clone was amplified with oligonucleotides specific for the relevant V, J, and C segments and the V-C products

Table 1. *Pairing of Vα24 with Vβ11 in Single Positive and DN T Cells*

Polyclonal lines:	Vα24+ CD4+		Vα24+ CD8+		Vα24+ DN	
Percent cells expressing:	α24	α24/β11	α24	α24/β11	α24	α24/β11
ALA	96	8	59	0	99	99
CDO	97	27	84	0	94	94
FOL	98	15	46	2	99	85
FSA	88	2	78	0	85	82
SDE	91	10	37	0	42	21

Vα24+CD4+, Vα24+CD8+, and VαCD4-8- cells were sorted from PBMC of five normal donors, expanded in culture for 6 d and analyzed by FACS® for the expression of the Vα24 and Vβ11 as in Fig. 1. In all donors α/β DN cells did not exceed 2% of peripheral blood mononuclear cells.



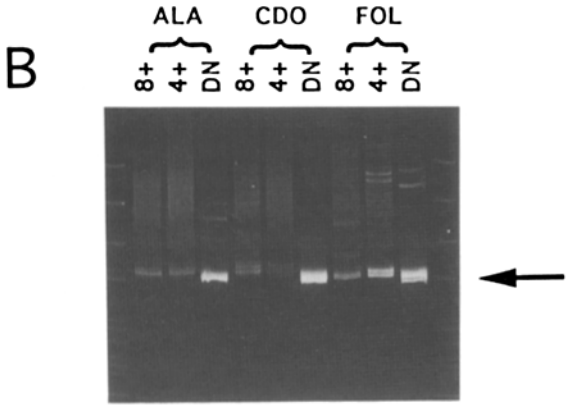


Figure 2. The Vα24 chain that pairs with Vβ11 in the α/β DN subset is homogeneous and identical to the invariant VαCO9. (*A*) RNA was extracted from short-term lines derived from three of the five donors described in Table 1 and reverse transcribed into cDNA. The cDNA was amplified with primers specific for Vα24 and Cα. The PCR products were hybridized with labeled oligonucleotides specific for Cα, JαQ, and the invariant Vα24–JαQ junctional sequence of clone CO9 (N-CO9). Controls include CO9 and T6, a CD4+ clone that expresses Vα24 joined to JαQ by a short N region (17). (*B*) The same PCR products as in *A* were subjected to heteroduplex analysis on native acrylamide gels. The *arrow* indicates the migration of the homoduplex bands, which were eluted from the gel and directly sequenced. The sequence was identical to that of CO9 (see also Table 2).

Table 2. *TCR Structure of Vα24⁺/Vβ11⁺ T Cell Clones*

Phenotype	Clonotype frequency	TCR structure		
Donor 1				
		valser Vα24-GTGAGC		asparggly GACAGAGGC-JαQ
DN	16/22			
		serserglu Vβ11-AGCAGTGAAT	serglygly CGGGGGG	asnthrglu GAACACTGAA-Jβ1.1
		valser Vβ24-GTGAGC	alagly GCGGGT	glyglyser GGAGGAAGC-JαT
CD4⁺	1/22			
		Vβ not 11		**Jβ not 2.1**
		valser Vα24-GTGAGC	glygly GGGGGA	serglygly TCAGGAGGA-JαW
CD4⁺	1/22			
		Vβ not 11		**Jβ not 2.1**
Donor 2				
		valser Vα24-GTGAGC		asparggly GACAGAGGC-JαQ
DN	7/26			
		serserglutyr Vβ11-AGCAGTGAATA	lysthrsergly TAAGACTAGCGGGC	protyrasnglu CCTACAATGAG-Jβ2.1
		valser Vα24-GTGAGC		asparggly GACAGAGGC-JαQ
DN	3/26			
		serserglu Vβ11-AGCAGTGA	pheglyglylys GTTCGGTGGAAA	asnthr GAACACC-Jβ2.2
		Vα24		**Jα not Q**
CD8⁺	1/26			
		serserglu Vβ11-AGCAGTGAA	glyalaalaglytrp GGGGCGGCCGGGTGG	gluthrgln GAGACCCAG-Jβ2.5
		val Vα24-GTG	alaarg GCCCGA	asparggly GACAGAGGC-JαQ
CD8⁺	2/26			
		serserglu Vβ11-AGCAGTGAA	serglyglyglyargser TCTGGAGGGGGGGAGGT	aspthr CAGATACG-Jβ2.3
CD4⁺	12/26	**Vα24** **Vβ11**		**Jα not Q** **Jβ not 2.1**
CD4⁺	2/26	**Vα24**		**Jα not Q**
		serarg Vβ11 AGCAG	glyserthrglymetser GGGGTCAACAGGGATGT	glyglu CCGGGGAG-Jβ2.2

Vα24⁺ T cells from donor 1 (1.1% α/β DN T cells) or Vα24⁺/Vβ11⁺ T cells from donor 2 (1.5% α/β DN T cells) were sorted and immediately cloned by limiting dilution. RNA from the clones was reverse transcribed into cDNA and amplified by PCR. The primers were chosen to recognize the TCR of the α/β DN clone CO9. Where indicated, the PCR product was purified from the agarose gel and directly sequenced. Underlined are residues that may be encoded by Dβ1 or Dβ2. The Vβ11 germ line encoded nucleotides have been identified on the basis of the germ line sequence provided by Dr. Leroy Hood (University of Washington, Seattle, WA).

were sequenced. Table 2 summarizes the results of this analysis in two different healthy donors with normal levels of α/β DN cells. In donor 1, all DN clones showed the same TCR-α and -β sequence. The fact that the β chain carries the same N region indicates that all the clones derive from a single T cell that has expanded in vivo. In the second donor two expanded DN clonotypes were detected, each with a characteristic N region and Jβ. Finally, all the three DN clonotypes carry the invariant CO9 α chain.

T cell clones carrying Vα24 and Vβ11 could also be found among $CD4^+$ and $CD8^+$ T cells. However, in this case the Vα24 carries N region or is rearranged to Jα segments other than JαQ (Table 2).

In conclusion, the use of mAbs and T cell clones allowed to identify an almost invariant TCR expressed on expanded clones of α/β DN T cells in all individuals tested. This receptor consists of an invariant Vα24–JαQ with no N region nucleotides, paired with Vβ11 bearing chains that carry N regions of different lengths and sequence and can use different Jβ segments.

There is a striking similarity between the invariant human Vα24–JαQ chain and the mouse Vα14-Jα281 described by Taniguchi et al. (20). Both lack junctional diversity and are highly homologous (62% aminoacid homology in the V region and 9 out of 10 identical residues in CDR3; reference 21). It is tempting to speculate that the lack of N region may be related to a late Vα rearrangement occurring after loss of terminal transferase and possibly extrathymically, as it has been shown for the mouse Vα14 (20).

There are two mechanisms that may account for the frequent occurrence of a particular TCR. The first is that the TCR is frequently generated by an homology-mediated recombination event (22–26) or that the α/β pairing is forced by molecular constraints. The second mechanism is that rare clones carrying specific receptors are expanded by recognition of their specific ligand. Our data clearly point to the second possibility because there is no evidence of molecular constraints for Vα24 rearrangement and pairing and, most important, in every individual $V\alpha24^+$ DN T cells derive from a single or a few expanded T cell clones as demonstrated by γ rearrangements (11) and Vβ N region diversity (this study).

The clonal expansion of these cells clearly carries the hallmark of antigen-driven selection and implies that the selective antigen must be very similar in all individuals and thus, most likely, not polymorphic (27). Although this ligand has not been characterized yet, it may be an antigen bound to a nonpolymorphic antigen-presenting molecule such as CD1, as described by Brenner et al. (9).

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Note added in proof: In this issue, Lantz and Bendelac (28) describe in the mouse a $CD4^-8^-$ T cell population that uses an invariant TCR Vα14–Jα281 chain strikingly homologous to the human invariant Vα24–JαQ.

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