Selective Reduction and Recovery of Invariant Vα24JαQ T Cell Receptor T Cells in Correlation with Disease Activity in Patients with Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** To study the regulatory role of CD4–CD8– double-negative (DN) invariant T cell receptor (TCR) Vα24JαQ T cells, a human counterpart of murine NK1+ T cells, in the autoimmune process of systemic lupus erythematosus (SLE).

Methods. We carried out a 2 step frequency analysis of DN Vα24JαQ T cells in patients with SLE before and after prednisolone therapy; the frequency of DN Vα24+ T cells was determined by 3 color FACS analysis and subsequently the frequency of Vα24JαQ rearrangement among DN Vα24+ T cells was determined by sequencing.

Results. DN Vα24+ T cells were significantly increased in patients with active SLE compared to healthy subjects. In healthy subjects, invariant Vα24JαQ TCR dominated in DN Vα24+ T cells at a high frequency (93–100%). However, the invariant Vα24JαQ TCR was not detected in DN Vα24+ T cells from patients with active SLE, and instead 2 to 9 Jα genes other than the invariant JαQ were oligoclonally expanded in the patients. In inactive SLE induced by prednisolone therapy, the invariant Vα24JαQ TCR could be detected in DN Vα24+ T cells from all the patients and dominated in most of the patients. Further, oligoclonally expanded Vα24+ clones other than the invariant JαQ gene in active disease states were significantly decreased by prednisolone therapy.

Conclusion. The selective reduction of DN invariant Vα24JαQ T cells is related to the disease progression of SLE, while DN TCR Vα24 T cells other than Vα24JαQ T cells constitute autoaggressive T cells in SLE. (J Rheumatol 2001;28:275–83)

Key Indexing Terms:
INVARIANT Vα24JαQ TCR T CELLS
SYSTEMIC LUPUS ERYTHEMATOSUS
CD4–CD8– DOUBLE-NEGATIVE T CELLS
CORTICOSTEROIDS

Murine NK1+ T cells are a specialized subset of CD4–CD8– double-negative (DN) T cell receptor-αß (TCR-αß) T cells that express the NK1 antigen, a member of the family of NKR-P1 natural killer cell receptors[1-4]. Moreover, a subpopulation of CD4+ T cells also expresses the NK1 antigen on the cell surface in mice. These NK1+ T cells have unusual features in comparison with the mainstream T cells and may play an important role in the regulation of some immune responses. First, NK1+ T cells possess an invariant TCR Vα14Jα281 that preferentially pairs with Vß8.2, Vß7, and Vß2[5,6]. This highly restricted TCR on NK1+ T cells presumably recognizes a monomorphic MHC class I-like molecule CD1d, rather than polymorphic MHC molecules[7]. NK1+ T cell development has recently been shown to be impaired in CD1 deficient mice[8-10]. Second, NK1+ T cells can promptly produce large amounts of interleukin 4 (IL-4) and interferon-γ (IFN-γ) by stimulation with anti-CD3 antibody[11-13], and this cytokine secretion is impaired in CD1 deficient mice lacking NK1+ T cells[8-10]. Furthermore, NK1+ T cells are decreased in correlation with the disease activity in autoimmune-prone mice[14-16] and have been suggested to regulate the autoimmune process in murine models of lupus erythematosus[14,15]. This is also supported by the acceleration of autoimmune symptoms by *in vivo* depletion of NK1+ T cells with anti-Vα14 antibody in lpr mice[15].

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DN invariant Vα24JαQ T cells are thought to be a human counterpart of murine NK1+ T cells[17-20]. The TCR Vα24JαQ chain has a high homology with murine Vα14Jα281 chain in both the amino acid and nucleotide sequences[17-20]. The Vß chains pairing with the Vα24JαQ are Vß11 and Vß13, which also have a high homology with murine Vß8 and Vß7[19,20]. It has recently been shown that human DN T cell clones bearing the invariant Vα24JαQ TCR also recognize CD1d molecule[21]. Moreover, DN Vα24JαQ T cells express NKR-P1 molecule on the surface[22]. Furthermore, DN Vα24JαQ T cells have been shown to produce both IL-4 and IFN-γ upon TCR stimulation[21-23]. However, the regulatory role of invariant Vα24JαQ DN T cells (human NK1+ T cells) in the autoimmune process of systemic lupus erythematosus (SLE) has not yet been clarified.

We analyzed the frequency of invariant Vα24JαQ DN T cells in patients with SLE in correlation with the disease activity before and after prednisolone therapy. We also studied the clonality of TCR Vα24 genes in DN αß T cells from patients with SLE by analyzing the nucleotide sequences of complementarity determining region 3 (CDR3) of TCR Vα24 genes.

Our results indicate that invariant Vα24JαQ DN T cells are closely related to the disease progression of human SLE, as indicated by the selective reduction of DN Vα24JαQ T cells in active SLE and the recovery of those cells in inactive SLE induced by prednisolone therapy. On the other hand, we also demonstrate that DN TCR Vα24 T cells other than Vα24JαQ T cells are oligoclonally expanded in active SLE and disappear in inactive SLE, suggesting that those cells constitute autoaggressive T cells in SLE.

MATERIALS AND METHODS

Patients. Five patients diagnosed with SLE[24] (all women, aged 23 to 55 yrs) were studied in the active state of SLE and also in their inactive states induced by prednisolone therapy (Table 1). SLE disease activity was evaluated according to the SLE Disease Activity Index (SLEDAI)[25], and the mean score before therapy was 17.6, range 12 to 26. The 5 patients with active SLE were treated with prednisolone at the initial dose of 1 mg/kg/day for 4 to 6 weeks, and then the dosage was gradually reduced as the levels of anti-DNA antibody and complements and other disease activity indexes improved with therapy. After 4 to 5 months of prednisolone therapy (20 to 25 mg/day at that time), the second evaluation was performed when their SLE became inactive and the mean SLEDAI score was 1.6. Five healthy subjects were also examined as controls.

Flow cytometry. Peripheral blood lymphocytes (PBL) were isolated from 20 ml of heparinized peripheral venous blood of 5 patients with SLE and 5 healthy subjects by Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) density gradient centrifugation.

Cells (1×10^6) were stained with fluorescence or biotin conjugated antibodies in phosphate buffered saline (PBS) containing 1% fetal calf serum for 30 min at 4°C. The following fluorescein isothiocyanate (FITC), phycoerythrin (PE), or biotin conjugated monoclonal antibodies (Mab) were used: CD4 (Leu-3a), CD8 (Leu-2a), TCR-αß (Becton Dickinson, Mountain View, CA, USA), and TCR Vα24 (Cosmo Bio Co., Tokyo, Japan). Cells stained with biotinylated Mab were then incubated with streptoavidin PE or Tricolor (Caltag, San Francisco, CA, USA). Stained cells were resuspended in PBS containing 1% fetal calf serum and analyzed by FACScan (Becton Dickinson) using the Cell Quest program.

Purification of CD4–CD8– double-negative T cells. CD4–CD8– double-negative (DN) TCR-αß T cells were sorted from peripheral blood lymphocytes (PBL) of SLE patients and healthy subjects by FACStar (Becton Dickinson) using anti-CD4 plus anti-CD8 Mab. The yields of DN T cells were about 1×10^5.

Cloning and sequencing of cDNA encoding TCR Vα genes. Total RNA (0.1–1 µg) was prepared from sorted DN T cells by the method of acid guanidinium thiocyanate/phenol/chloroform extraction using Isogen solution (Nippon Gene Co., Tokyo, Japan). The first strand complementary DNA (cDNA) was then synthesized from 0.1–1 µg of total RNA in 20 ml of reaction buffer containing oligo-dT primer using avian myeloblastosis virus reverse transcriptase. The reaction mixture was incubated at 25°C for 10 min and then at 42° C for 60 min.

TCR Vα24 cDNA from DN T cells were amplified by polymerase chain reaction (PCR) using primers for Vα24 with an EcoRI restriction site (5'-CGAATTCCTCAGCGATTCAGCCTCCTAC-3') and Ca (5'-CGAATTCGGTGAATAGGCAGACAGACTT-3'). The denaturing step was done at 95°C for 1.5 min, the annealing step at 60°C for 1 min, and the extension step at 72°C for 1 min, for 30 cycles on a DNA thermal cycler (Perkin-Elmer Corp., Norwalk, CT, USA). PCR products were purified by phenol extraction, precipitated with ethanol, and digested with excess amounts of EcoRI. The DNA fragments with expected sizes of the cDNA were enriched by preparative low melting-point agarose gel electrophoresis. The recovered DNA fragments were ligated to M13mp19 plasmids obtained by EcoRI digestion. Phages were grown on TG-1 *Escherichia coli* cells. After hybridization with a Cα probe, a single phage was allowed to grow, and recombinant phage DNA was purified for DNA sequence determination. Sequencing reactions were performed by the dye primer method using an automated sequencer (Applied Biosystems).

Plaque hybridization. TCR Vα24 cDNA libraries were generated by PCR using RNA from the DN T cells with primers for the Vα24 and Cα. Recombinant plaques were transferred from DYT plates to 2 nitrocellulose

Table 1. Patient profiles in active SLE and inactive SLE induced by prednisolone therapy.

SLE Patients	Age	Sex	Active SLE			Inactive SLE			
			C3 (mg/dl)	Anti-DNA (U/ml)	SLEDAI (score)	C3 (mg/dl)	Anti-DNA (U/ml)	SLEDAI (score)	PSL (mg/day)
1	29	F	43	15	12	56	0	0	20
2	55	F	17	456	26	51	5	4	20
3	35	F	26	26	24	54	0	4	25
4	23	F	37	533	12	51	0	0	25
5	28	F	31	7	14	52	0	0	25

SLEDAI: SLE Disease Activity Index; PSL: prednisolone.

membranes. These were hybridized with either a ^{32}P labeled Vα24 probe (5'-CTCAGCGATTCAGCCTCCTAC-3') or a 53 bp JαQ probe. The latter probe was synthesized by PCR with 5'-JαQ (5'-CAACCCTGGGGAGGC-TATAC-3') and 3'-JαQ (5'-AGGCCAGACAGTCAACTGAG-3') primers and purified by electroelution.

Data analysis. Data are summarized as mean ± standard deviation (SD). The statistical analysis of the results was performed by the unpaired and paired t test. P values < 0.05 were considered significant.

RESULTS

Increase of DN Vα24+ T cells in peripheral blood from SLE patients. To determine the involvement of invariant TCR Vα24JαQ T cells in the pathogenesis of SLE, we first counted the number of CD4–CD8– double-negative TCR-αß T cells and DN TCR Vα24+ T cells in PBL from 5 patients with active SLE and 5 healthy subjects by flow cytometry (Figure 1). As shown in Table 2, the percentage of DN αß T cells in PB from SLE patients was greater than that from healthy subjects (controls 1.08 ± 0.24% vs SLE 2.08 ± 0.48%, mean ± SD, n = 5; p < 0.01), consistent with a previous study showing that DN TCR-αß T cells were markedly expanded in patients with active SLE[26]. The number of DN Vα24+ T cells was also significantly increased in PB of SLE patients compared to healthy subjects (controls 3.86 ± 0.63/mm^3 vs SLE 7.73 ± 2.49/mm^3; p < 0.01). In healthy subjects, 20.1% (17 to 24%) of DN αß T cells were Vα24 positive, while 30.6% (23 to 40%) of DN αß T cells expressed TCR Vα24 in SLE patients (Table 2). In addition, DN αß T cells other than Vα24+ T cells expressed various Vα chains in healthy controls and patients with active or inactive SLE (data not shown).

Selective reduction of TCR Vα24JαQ T cells in SLE patients. To determine the clonality of TCR Vα24 genes in DN αß T cells from SLE patients, we analyzed the

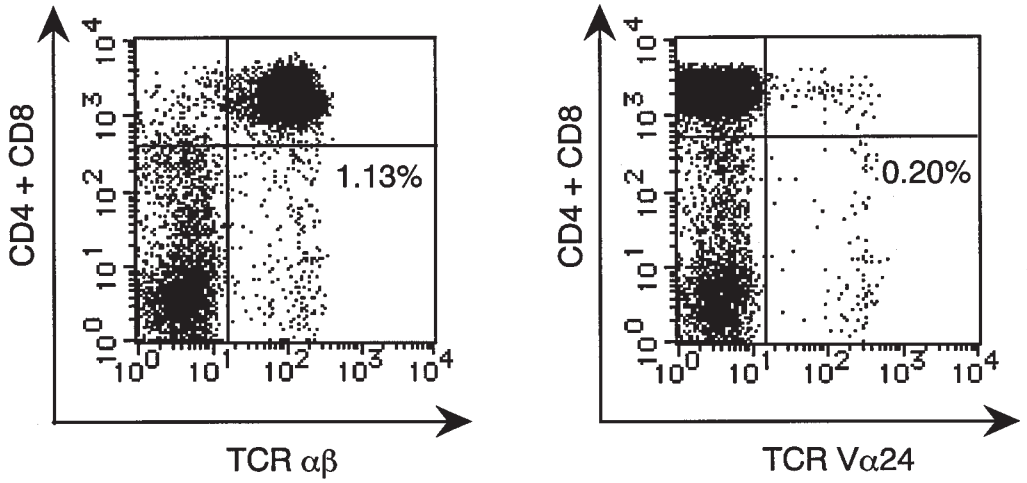

Figure 1. FACS profiles of CD4–CD8– double negative (DN) TCR-αß T cells and DN TCR Vα24+ T cells in peripheral blood of a healthy subject. DN TCR-αß T cells (left) and DN TCR Vα24+ T cells (right) were analyzed by FACS using PE conjugated anti-CD4 plus anti-CD8 Mab and FITC conjugated anti-TCR-αß Mab or anti-Vα24 Mab.

Table 2. Double-negative TCR Vα24+ T cell population in PBL from patients with active SLE. DN TCR αß T cells and DN TCR Vα24+ T cells in PBL from healthy controls and patients with active SLE were analyzed by FACS using PE conjugated anti-CD4 plus anti-CD8 Mab and FITC conjugated anti-TCR αß Mab or anti-Vα24 Mab.

	Healthy Controls	SLE Patients	
		Active	Treated
	(n = 5)	(n = 5)	(n = 5)
PBL (/mm^3)	1825 ± 262	1276 ± 416	1450 ± 577
DN αß T cells/PBL (%)	1.08 ± 0.24	2.08 ± 0.48*	1.51 ± 0.18
DN αß T cells (/mm^3)	19.28 ± 2.15	27.24 ± 8.07	22.09 ± 7.89
DN Vα24 T cells/PBL (%)	0.22 ± 0.05	0.61 ± 0.05**	0.35 ± 0.05*
DN Vα24 T cells (/mm^3)	3.86 ± 0.63	7.73 ± 2.49**	5.35 ± 2.57
DN Vα24/DN αß T cells (%)	20.1 ± 2.9	30.6 ± 7.1*	23.6 ± 2.7*

*p < 0.05, **p < 0.01, significantly different from the mean value of healthy subjects.
PBL: peripheral blood lymphocytes; DN: CD4–CD8– double-negative.
Data are mean ± SD.

Table 3. Junctional sequences of TCR Vα24 genes obtained from DN αß T cells in patients with inactive SLE after prednisolone therapy. TCR Vα24 cDNA clones were randomly isolated from PCR amplified libraries of DN T cells from patients with inactive SLE after prednisolone therapy, and were sequenced. Nucleotide sequences of the 3′ of TCR Vα, N region, and the 5′ of the Jα region are aligned. The frequency of identical sequences defined is shown in the right column.

Materials	Vα	N	Jα	Frequency
	93		104	
SLE-1				
	GTGGTGAG	TCCGAAGACC	CAGTTC JαU	8/10
	GTGGTGA	ACTCCCCAAAGAGGGGA	GGAGGAAACAAACTC JαAD210	1/10
	GTGGTGA	CC GCATCAGGAGGAAGCTACATACCT	JαHAP51G	1/10
SLE-2				
	GTGGTG	GAA GCTGCAGGCAACAAGCTA	JαAC25	3/11
	GTGGTGAGC	GGGGGGGGGAAGGAATAT	GGAAACAAGCTG IGRJα09	3/11
	GTGGTGA	TCAGGATT TCAGGATACAGCACCCTC	JαAD17	2/11
	GTGGTGAGC	GTGGGC GCTGGTGGTACTAGCTATGGAAAGCTG	JαAB21	1/11
	GTGGTGAGC	GCGAGTGTTA ATAACAATGACATG	IGRJα10	1/11
	GTGGTG	GTCCCCGAGTA TAACACCGACAAGCTC	JαI	1/11
SLE-3				
	GTGGTGAGC	GACC CAGGCAAATCA	IGRJα01G	9/11
	GTGGTGAG	ACTCAACCAGGC AGGAACTGCTCTG	JαAC112	2/11
SLE-4				
	GTGGTG	CCCCTCCTTGGGGATC GTGGCTACAATAAGCTG	IGRJα08	7/11
	GTGGTGAGC	GCGGGAT ATGGAGGAAGCCAAGGAAATCTC	IGRJα06	4/11
SLE-5				
	GTGGTGA	AG GGGAACAACAGACTC	IGRJα11	2/12
	GTGGTGAGC	G CAGGATACAGCACCCTC	JαAD17	2/12
	GTGGTGAGC	GGGGGTTACCAGAAAGTT	JαHAP23G	2/12
	GTGG	CACAC GGGAACAACAGACTC	IGRJα11	1/12
	GTGGTGAGC	GTGGGC GACTACAAGCTC	JαN	1/12
	GTGGT	ATAC AACTTCAACAAATTTTAC	JαAC24	1/12
	GTGGTGAGC	CAGTGGATGGAT AGCAGCTATAAATTG	JαAB17	1/12
	GTGGTGAG	TGA ATCAGGAGGAAGCTACATACCT	JαHAP51G	1/12
	GTGGTGA	CCCCCCATTT CTGGTGGCTACAATAAGCTG	IGRJα08	1/12
Cont-1				
	GTGGTGAGC	GACAGAGGCTCAACCCTGGGGAGGCTA	JαQ	14/15
	GTGGTG	GTACACA CCGGTAACCAGTTC	JαU	1/15
Cont-2				
	GTGGTGAGC	GACAGAGGCTCAACCCTGGGGAGGCTA	JαQ	15/16
	GTGGTGAGC	CGGGAAG CTGGCAACAACCGTAAGCTG	IGRJα13	1/16
Cont-3				
	GTGGTGAGC	GACAGAGGCTCAACCCTGGGGAGGCTA	JαQ	14/14
Cont-4				
	GTGGTGAGC	GACAGAGGCTCAACCCTGGGGAGGCTA	JαQ	11/11
Cont-5				
	GTGGTGAGC	GACAGAGGCTCAACCCTGGGGAGGCTA	JαQ	15/15

nucleotide sequences of CDR3 of TCR Vα24 genes in peripheral blood DN T cells of healthy subjects and SLE patients. cDNA encoding Vα24 genes from DN T cells were amplified by PCR, cloned, and sequenced.

In healthy subjects, invariant Vα24JαQ TCR dominated in DN Vα24+ T cells of all 5 individuals at a high frequency (14/15, 15/16, 14/14, 11/11, and 15/15) (Table 3). In 3 of 5 individuals, only the invariant JαQ gene was detected in Vα24 cDNA clones from DN T cells. In 2 other subjects (control 1 and control 2), in addition to the JαQ gene, another Jα gene, JαU, and IGRJα13 were detected in DN Vα24+ T cells at a frequency of 1/15 and 1/16, respectively.

On the other hand, the invariant Vα24JαQ TCR most dominantly detected in healthy subjects was not detected in any patients with active SLE (n = 5), and instead 2 to 9 Jα genes other than the invariant JαQ were detected in Vα24 cDNA clones of the patients (Table 3). Further, oligoclonal expansion of other Vα24 TCR was observed in 3 of 5 SLE patients. JαU in SLE Patient 1, IGRJα01G in SLE Patient 3, and IGRJα08 and IGRJα06 in SLE Patient 4 dominated in DN Vα24+ T cells at a high frequency (8/10, 9/11, and 7/11 and 4/11, respectively) (Table 3). In two other patients, Patient 2 and Patient 5, heterogeneous Jα genes were detected, but there was some accumulation of the same clones, such as JαAC25, IGRJα09, JαAD17, IGRJα11, and JαHAP23G. Thus, the dominant Vα24 TCR clones differed among the patients with active SLE, and no shared amino acid sequence was observed.

To further determine whether the invariant Vα24JαQ T cells are decreased in DN T cells from SLE patients, cDNA libraries generated by PCR from DN T cells were hybridized with the Vα24 probe and the JαQ probe. Frequencies of the invariant Vα24JαQ TCR among total Vα24 TCR clones were estimated by the number of positive plaques and expressed as the ratio of the invariant Vα24JαQ to total Vα24. As shown in Table 4, the invariant JαQ gene was hardly detected in DN Vα24+ T cells from all SLE patients (0.77 ± 0.92%, n = 5; $p < 0.001$), while DN Vα24+ T cells from healthy subjects mostly used the invariant JαQ TCR at a high frequency (80.2 ± 10.3%, n = 5). Indeed, the absolute cell number of DN T cells bearing the invariant Vα24JαQ was drastically decreased in SLE patients compared to healthy controls (controls 3.07 ± 0.43/mm^3 vs SLE 0.066 ± 0.089/mm^3; $p < 0.001$) (Table 4).

Recovery of invariant Vα24JαQ T cells by prednisolone therapy in SLE patients. To determine whether Vα24JαQ T cells are related to disease activity of SLE, we examined the clonality of DN Vα24+ T cells from the same 5 SLE patients in their inactive disease states induced by prednisolone therapy. The 5 patients with active SLE were treated with prednisolone at the initial dose of 1 mg/kg/day for 4 to 6 weeks, and then the dosage was gradually tapered as the levels of anti-DNA antibody and complements and other disease activity indexes[25] improved with the therapy.

Table 4. Frequencies of invariant Vα24JαQ DN T cells in patients with active SLE.

Source	Vα24JαQ/Vα24* %	DN Vα24+ T Cells† (/mm^3)	DN Vα24JαQ T Cells (/mm^3)
Control			
1	236/349 (67.6)	4.93	3.33
2	339/481 (70.5)	3.30	2.33
3	500/570 (87.7)	3.60	3.16
4	210/241 (86.7)	3.68	3.19
5	235/265 (88.7)	3.78	3.35
SLE patient			
1	2/389 (0.51)	7.15	0.03
2	0/203 (0.00)	5.28	0
3	1/155 (0.64)	11.84	0.07
4	0/120 (0.00)	6.44	0
5	8/294 (2.72)	7.93	0.21

*TCR Vα24 cDNA libraries generated by PCR from DN T cells were blotted on 2 separate filters and hybridized with either Vα24-specific oligonucleotide probe or JαQ probe. The ratio of invariant Vα24JαQ/Vα24 cells was calculated from the number of positive plaques.
†DN TCR Vα24+ T cells in PBL from healthy controls and patients with active SLE were analyzed by FACS using PE conjugated anti-CD4 plus anti-CD8 Mab and FITC conjugated anti-Vα24 Mab.
PBL: peripheral blood lymphocytes, DN: CD4– CD8– double-negative.

In the inactive state of SLE, the invariant Vα24JαQ gene was detected in DN Vα24+ T cells from all 5 SLE patients treated with prednisolone and dominated in 4 of 5 patients (Table 5). In one patient, Patient 5, all of 14 Vα24 cDNA clones were the invariant JαQ gene. In 3 other patients, Patients 2, 3, and 4, the invariant JαQ gene also dominated in DN Vα24+ T cells at a frequency of 14/20, 14/17, and 8/15, respectively.

The recovery of the invariant Vα24JαQ DN T cells in SLE patients treated with prednisolone was also revealed by plaque hybridization assay. The frequency of the invariant JαQ gene in total Vα24+ clones from DN T cells was significantly increased by prednisolone therapy (64.9 ± 21.3%, n = 5; $p < 0.001$) (Table 6). Further, the cell number of the invariant Vα24JαQ DN T cells recovered to the normal range of healthy subjects with prednisolone therapy (3.63 ± 2.35/mm^3; $p < 0.01$) (Table 6 and Figure 2).

It is also noteworthy that oligoclonally expanded Vα24+ clones other than the invariant JαQ gene in active disease states were significantly decreased by prednisolone therapy (Table 5). The predominant usages of JαU in SLE Patient 1 and IGRJα08 and IGRJα06 in Patient 4 were not affected by the therapy. In Patient 3, the frequency of the predominant JGRJα01G was decreased from 9/11 to 1/17 by the therapy. In addition, DN Vα24+ T cells were also significantly decreased by prednisolone therapy (before prednisolone therapy 7.73 ± 2.49/mm^3 vs after the therapy 5.34 ± 2.56/mm^3; $p < 0.002$) (Table 6 and Figure 2).

Table 5. Junctional sequences of TCR Vα24 genes obtained from DN cells in patients with active SLE. TCR Vα24 cDNA clones were randomly isolated from the PCR amplified libraries of DN T cells from patients with active SLE (n = 5) and healthy controls (cont) (n = 5) and were sequenced. Nucleotide sequences of the 3′ of TCR Vα, N region, and the 5′ of the Jα region are aligned. The frequency of identical sequences is shown in the right column.

Materials	Vα	N	Jα		Frequency
	93		104		
SLE-1					
	GTGGTGA	CCGCCAT	CTCAGGAACCTACAAATAC	JαAP511	4/16
	GTGGTGAGC	GACAGAGGCTCAACCCTGGGGAGGCTA		JαQ	3/16
	GTGGTGAGC	GCGTCGATCCCGATCA	AAGCTGCAGGCAACAAGCTA	JαAC25	3/16
	GTGGTG	GTCCCGGGGGG	CTACAAGCTC	JαN	3/16
	GTGGTGA	GGGGAGCCACGGCTTA	TAACACCGACAAGCTC	JαI	2/16
	GTGGTG	G	CTGGTGGCTACAATAAGCTG	IGRJα08	1/16
SLE-2					
	GTGGTGAGC	GACAGAGGCTCAACCCTGGGGAGGCTA		JαQ	14/20
	GTGGTGAGC	GCGAGAT	CTAACTTTGGAAATCAGAAATTA	JαAA13	3/20
	GTGGTGA	TCCC	TAACACCAATGCAGGCAAATCA	IGRJα01G	2/20
	GTGGTGAGC	GGAATGAGG	AACCAGGGAGGAAAGCTT	JαH	1/20
SLE-3					
	GTGGTGAGC	GACAGAGGCTCAACCCTGGGGAGGCTA		JαQ	14/17
	GTGGTGAGC	GTGGGC	GCTGGTGGTACTAGCTATGGAAAGCTG	JαJ	1/17
	GTGGTGAGC	C	CCTCAGGAACCTACAAATAC	JαAP511	1/17
	GTGGTGAGC	GACC	CAGGCAAATCA	IGRJα01G	1/17
SLE-4					
	GTGGTGAGC	GACAGAGGCTCAACCCTGGGGAGGCTA		JαQ	8/15
	GTGGTGAGC	GGTTATA	CCTCAGGAACCTACAAATAC	JαAP511	4/15
	GTGGTGAGC	AATAATAAT	GCAGGCAACATGCTC	JαAC17	1/15
	GTGGTGAGC	TTAGGGTCTAACGAC	TACAAGCTC	JαN	1/15
	GTGGTG	AGG	ACGGGAGAGGGAAACAAACTC	JaAD210	1/15
SLE-5					
	GTGGTGAGC	GACAGAGGCTCAACCCTGGGGAGGCTA		JαQ	14/14

Table 6. Frequencies of invariant Vα24JαQ DN T cells in patients with inactive SLE after prednisolone therapy.

Source	Vα24JαQ/Vα24* (%)	DN Vα24+ T Cells‡ (/mm^3)	DN Vα24JαQ T Cells (/mm^3)
SLE Patient			
1	77/239 (32.3)	4.07	1.32
2	219/137 (79.8)	3.97	3.17
3	160/216 (74.1)	9.77	7.24
4	156/286 (54.5)	3.57	1.95
5	234/280 (83.6)	5.36	4.48

*TCR Vα24 cDNA libraries generated by PCR from DN T cells were blotted on 2 separate filters and hybridized with either Vα24-specific oligonucleotide probe or JαQ probe. The ratio of invariant Vα24JαQ/Vα24 cells was calculated from the number of positive plaques.
‡DN TCR Vα24+ T cells in PBL from healthy controls and patients with active SLE were analyzed by FACS using PE conjugated anti-CD4 plus anti-CD8 Mab and FITC conjugated anti-Vα24 Mab.
PBL: peripheral blood lymphocytes, DN: CD4– CD8– double-negative.

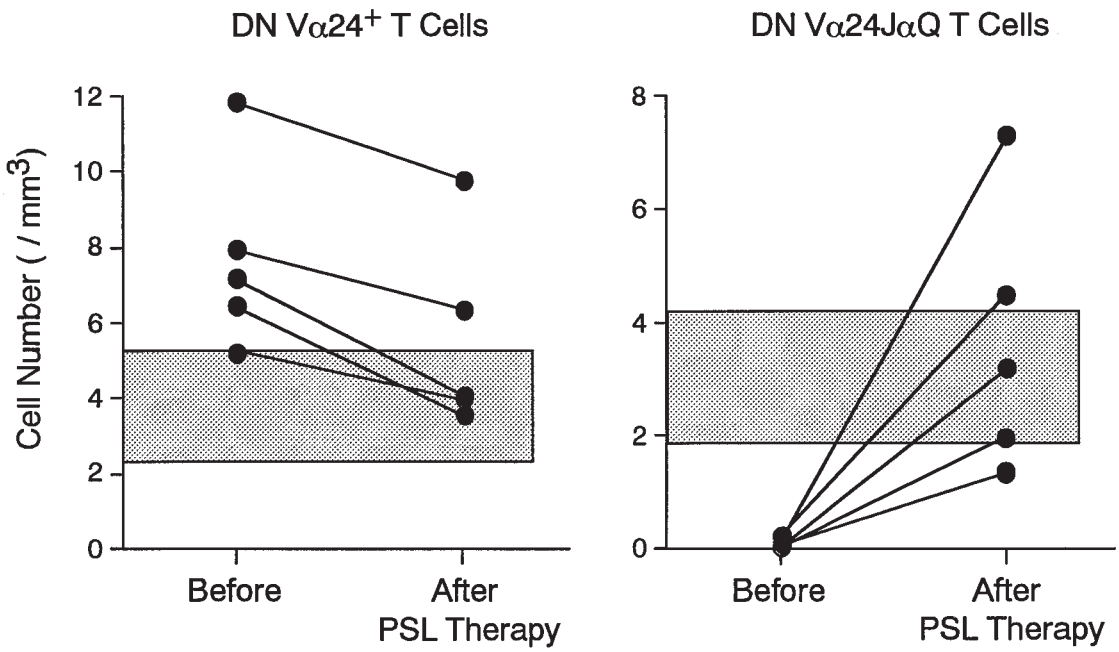

Figure 2. Cell number of DN Vα24+ T cells and DN invariant Vα24JαQ T cells in SLE patients before and after prednisolone therapy (PSL). Cell number of DN Vα24+ T cells (left) and DN invariant Vα24JαQ T cells (right) was assessed in 5 patients with SLE before and 4 to 5 months after prednisolone therapy as described in Tables 4 and 6. Shaded areas show the range (mean ± 2 SD) of cell number of DN Vα24+ T cells and DN Vα24JαQ T cells from 5 healthy donors.

DISCUSSION

Accumulating evidence indicates that DN invariant Vα24JαQ T cells are probably a human counterpart of murine NK1+ T cells[17-20]. In this study, we demonstrate that DN invariant Vα24JαQ T cells are related to the disease progression of human SLE, as indicated by the selective reduction of DN Vα24JαQ T cells in active SLE and the recovery of those cells in inactive SLE induced by prednisolone therapy. On the other hand, we also show that DN TCR Vα24 T cells rearranged to Jα genes other than the invariant JαQ gene are oligoclonally expanded in active SLE and disappear in inactive SLE, suggesting that those cells might function as autoaggressive T cells in SLE.

We show that invariant Vα24JαQ DN T cells are decreased in patients with active SLE and, for the first time, that the selective reduction of the invariant Vα24JαQ DN T cells in autoimmune diseases recovers to normal levels with corticosteroid therapy. We previously reported a selective reduction of invariant Vα24JαQ DN T cells in patients with systemic sclerosis[27]. Recently, Wilson, *et al*[28] also showed that invariant Vα24JαQ DN T cells were decreased in patients with type 1 diabetes, and the reduction of those cells was more severe in progressive subjects than nonprogressive subjects. However, the recovery of the invariant Vα24JαQ DN T cells in human systemic sclerosis or diabetes was not studied in their reports, because patients with these diseases are not usually treated with high doses of corticosteroid. Thus, our observations of the recovery of DN Vα24JαQ T cells in SLE patients with prednisolone therapy indicate that the decrease of DN Vα24JαQ T cells is not due to a genetic defect causing the lack of those cells in SLE. In contrast, it has been shown that SJL[29] and NOD[16] mice genetically lack NK1+ T cells even in young mice and have a marked propensity to autoimmune diseases, experimental allergic encephalitis[30], and diabetes[31], respectively.

We also show that DN T cells bearing Vα24 TCR other than Vα24JαQ are oligoclonally expanded in patients with active SLE. In addition, these Vα24 TCR sequences were not detected in healthy individuals. Thus, these findings suggest that the expanded oligoclonal Vα24 TCR in patients with active SLE could be induced by antigen driven stimulation and constitute autoaggressive T cells in autoimmune status. Sequence differences in the oligoclonal Vα24 TCR that dominate in SLE patients might reflect differences in the polymorphism of restriction elements or in epitope specificities. Our findings are consistent with studies showing that DN TCR-αß T cells that induced the production of pathogenic anti-DNA autoantibodies were markedly expanded in patients with active SLE, but not in normal subjects or SLE patients in remission[26]. It has recently been shown that anti-DNA autoantibody-inducing CD4+ T cells in SLE patients use the restricted TCR Vα gene, Vα8, at a high frequency that contains highly charged residues in their CDR3 loops[32]. Interestingly, these CD4+ T cells in SLE patients responded to charged epitopes in various DNA-binding nucleoproteins, such as high mobility group chro-

mosomal protein and nucleosomal histone proteins[32]. Therefore, it is possible that the oligoclonally expanded DN Vα24+ T cells may also recognize such DNA-binding nucleoproteins to help B cells produce the pathogenic autoantibodies.

Numerous abnormalities in cytokine production have been detected in patients with active SLE. Increased serum levels of IL-6[33-35] and IL-10[36] have been shown to be linked with the overproduction of pathogenic autoantibodies in patients with SLE. IL-10 and IL-6 producing cells are also increased in SLE patients[37]. In contrast, serum IL-2 levels are reduced in SLE patients[33,38], and the secretion of IL-2 and IFN-γ by cultured PBL from these patients is reduced[33,39-41]. Furthermore, disease severity of SLE correlated significantly with the ratio of IL-10 to IFN-γ secreting cells[37]. Therefore, increased production of IL-10 in SLE patients[36,37] may account for the decrease of DN Vα24JαQ T cells. Indeed, we found that the cell growth of DN Vα24JαQ T cells from healthy subjects was significantly inhibited by the addition of IL-10 into the culture (our unpublished data).

Decreased IFN-γ production in SLE patients[33,37,41] might also contribute to the decrease of DN Vα24JαQ T cells because IFN-γ upregulates CD1d expression[42] and the development of murine NK1+ T cells requires CD1d recognition through TCR[8-10]. Thus, the dysregulated cytokine production in SLE may account for the selective reduction of DN Vα24JαQ T cells.

In addition to an indirect effect on DN Vα24JαQ T cells through the inhibition of abnormal cytokine production by conventional T cells, corticosteroid may directly induce the increase of DN Vα24JαQ T cells, since NK1.1+ T cells were shown to be resistant to corticosteroid induced apoptosis compared with conventional T cells, and corticosteroid treatment increased the proportion of NK1.1+ T cells in spleen and liver in mice[43]. Further, Milner, *et al* recently demonstrated that corticosteroid enhanced anti-CD3 mediated proliferation of Vα24JαQ T cell clones, but suppressed the proliferation of CD4+ T cell clones in humans[44]. Thus, corticosteroid treatment could directly increase invariant Vα24JαQ DN T cells, but could decrease other oligoclonally expanded T cells in SLE.

The pathophysiologic role of NK1+ T cells in modulating autoimmune diseases has been suggested in mice. The reduction of NK1+ T cells in murine lupus has been reported to correlate with the disease activity in autoimmune prone mice[14,15]. Mieza, *et al*[15] showed that *in vivo* deletion of NK1+ T cells accelerated the development of autoimmune disease in lpr mice and the introduction of Va14Jα281 transgene delayed the onset of the disease, suggesting that NK1+ T cells play a role in regulating autoreactive T cells in the autoimmune process. However, CD1 deficient mice lacking NK1+ T cells did not develop autoimmune diseases[8-10]. Further, Vα14 NK1+ T cell deficient mice also showed no autoimmune features[45]. Collectively, NK1+ T cells are not essential for the prevention of autoimmune diseases, but modulate the progression of the disease.

Several mechanisms by which murine NK1+ T cells can modulate the progression of autoimmune diseases have been proposed. One possible mechanism is that NK1+ T cells produce large amounts of IL-4 and IFN-γ upon stimulation and thereby suppress the effects and development of autoaggressive T cells. IL-4 produced by NK1+ T cells suppresses the development and effects of Th1 type cells[46], which play important roles in autoimmune diseases[47] such as diabetes[48] and SLE[49]. IFN-γ may regulate autoreactive Th2 cells[46] that also contribute to the development of SLE[50]. Because we have shown that invariant Vα24JαQ DN T cells predominantly produce IFN-γ[51], the decrease of these cells may contribute to the progression of SLE through the decreased IFN-γ production. Second, NK1+ T cells could also delete autoaggressive T cells, since NK1+ T cells exert prominent cytotoxic activity in both Fas ligand dependent and independent fashions[45,52].

In summary, we have shown that the selective reduction and recovery of DN invariant Vα24JαQ T cells is related to the disease progression of SLE, while DN TCR Vα24 T cells other than Vα24JαQ T cells constitute autoaggressive T cells in SLE.

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