

Analysis of $\gamma\delta$ T-cell receptor repertoire in the human nasal mucosa

Kazuhiko Takeuchi, MD, Noriko Hirata, MD, Kotaro Ukai, MD, and
Yasuo Sakakura, MD *Mie, Japan*

$\gamma\delta$ T cells play important roles as the first line of defense in mucosal immunity. As a feature of the human $\gamma\delta$ T-cell receptor (TCR) repertoire, highly selective V-J gene usage is observed for γ and for δ , and preferential V-J γ and V-D-J δ pairing occurs.¹ In human beings, for example, 75% of $\gamma\delta$ T cells in most adult peripheral blood cells express V γ 2-J γ 1.2/V δ 2-J δ 1, and 25% express V γ 1-J γ 2/V δ 1-J δ 1.¹ The $\gamma\delta$ TCR repertoire analysis of normal skin indicated the presence of V δ 2-J δ 1 rearrangements with minor V δ 1-J δ 1 products.² $\gamma\delta$ Intraepithelial lymphocytes in the small intestine and colon express predominantly V δ 1-J δ 1.¹ Like these, certain receptors predominate at given anatomic locations.

Only a few studies have been done on intraepithelial lymphocytes in the nose. To elucidate the $\gamma\delta$ TCR repertoire in the nose, we performed reverse-transcription polymerase chain reaction (RT-PCR) analysis by using nasal epithelial samples obtained from normal subjects. We determined which V γ and V δ families are used and which J δ family is rearranged to V δ families.

METHODS

To obtain samples of normal nasal mucosa, the surface of the inferior turbinate of either side was scraped with a curette in 12 volunteers (19 to 32 years of age, all men) who had normal rhinoscopy results and had neither significant nasal complaints at the time of sampling nor a history of nasal diseases. Immediately after samples of nasal mucosa were obtained, the samples were suspended in denaturing solution. After vortexing the mixtures, we followed a single-step method of RNA isolation described by Chomczynski and Sacchi.³ Each RNA, prepared as described above, was reverse-transcribed into complementary DNA by using oligo(dT) as a primer. Five microliters of diluted cDNA⁴ was used for each PCR amplification. Before $\gamma\delta$ PCR analysis, β -actin cDNA was amplified by using the two primers: 5'-ATGGATGATGATATCGCCGCG-3' (sense) and 5'-ATGTCGTCCAGTTGGTGACGAT-3' (antisense), which provided products of 246 bp. PCR amplification and Southern blot analysis of $\gamma\delta$ TCR was performed as described by Wucherphennig et al.⁴ As a positive control, we used cDNA prepared from nasal polyp RNA, which had previously been

Abbreviations used

PBL:	Peripheral blood leukocyte
TCR:	T-cell receptor
RT-PCR:	Reverse-transcription polymerase chain reaction
V, D, J:	Variable, Diversity, Joining

confirmed to contain all the transcripts. Human peripheral blood leukocyte cDNA (Clontech, Palo Alto, Calif.) was also analyzed for comparison.

RESULTS

Amplification of V γ 1 to V γ 4 gene segments from normal human nasal mucosa cDNA resulted in bands for V γ 1, V γ 2, and V γ 3 reactions (Fig. 1, A). In about half of the specimens, V γ 1 and V γ 3 amplifications produced equally strong signals. As for amplification of the TCR δ chain, V δ 1 amplification produced the strongest signal in seven samples (66.7%, Fig. 1, A), indicating that the majority of TCR δ chains in the nose are rearranged to V δ 1. Intensity of each β -actin band was comparable (Fig. 1, A).

To determine J δ gene usage of human nasal TCR δ chains, three sets of nitrocellulose filters for four nasal samples, which had produced distinct bands when a C δ internal probe was used, as well as peripheral blood leukocytes (PBLs), were prepared and hybridized with probes for J δ 1, J δ 2, and J δ 3 gene segments. In all three specimens in which V δ 1 was expressed, hybridization of a V δ 1-C δ gene segment to a J δ 1 gene segment produced strong signals (Fig. 1, B). In contrast, a product from a V δ 2-C δ amplification with cDNA from PBLs produced the strongest hybridization with a J δ 1 probe. Weaker signals were detected by using probes for J δ 2 and J δ 3 in the V δ 2-C δ amplification product in PBLs.

DISCUSSION

This study clearly indicates that V δ 1 is preferentially utilized and rearranged to J δ 1 in the nasal mucosa in most healthy individuals. The predominance of V δ 1-J δ 1-bearing T cells in the intestine,¹ gingiva,⁵ and nose and the structural similarity of these organs suggests that the V δ 1 $\gamma\delta$ T cells may be specific to intraepithelial lymphocytes in the mucosa.

There is a specific feature in V δ 1-bearing T cells. The V δ 1 cells are extremely adherent to plastic surfaces. Recently, Nakajima et al.⁶ reported that $\gamma\delta$ T

From the Department of Otorhinolaryngology, Mie University School of Medicine.

Received for publication Nov. 9, 1995; revised June 25, 1996; accepted for publication July 11, 1996.

Reprint requests: Yasuo Sakakura, MD, Department of Otorhinolaryngology, Mie University School of Medicine, 2-174 Edobashi, Tsu, Mie 514, Japan.

J Allergy Clin Immunol 1997;99:251-3.

Copyright © 1997 by Mosby-Year Book, Inc.

0091-6749/97 \$5.00 + 0 1/1/76579

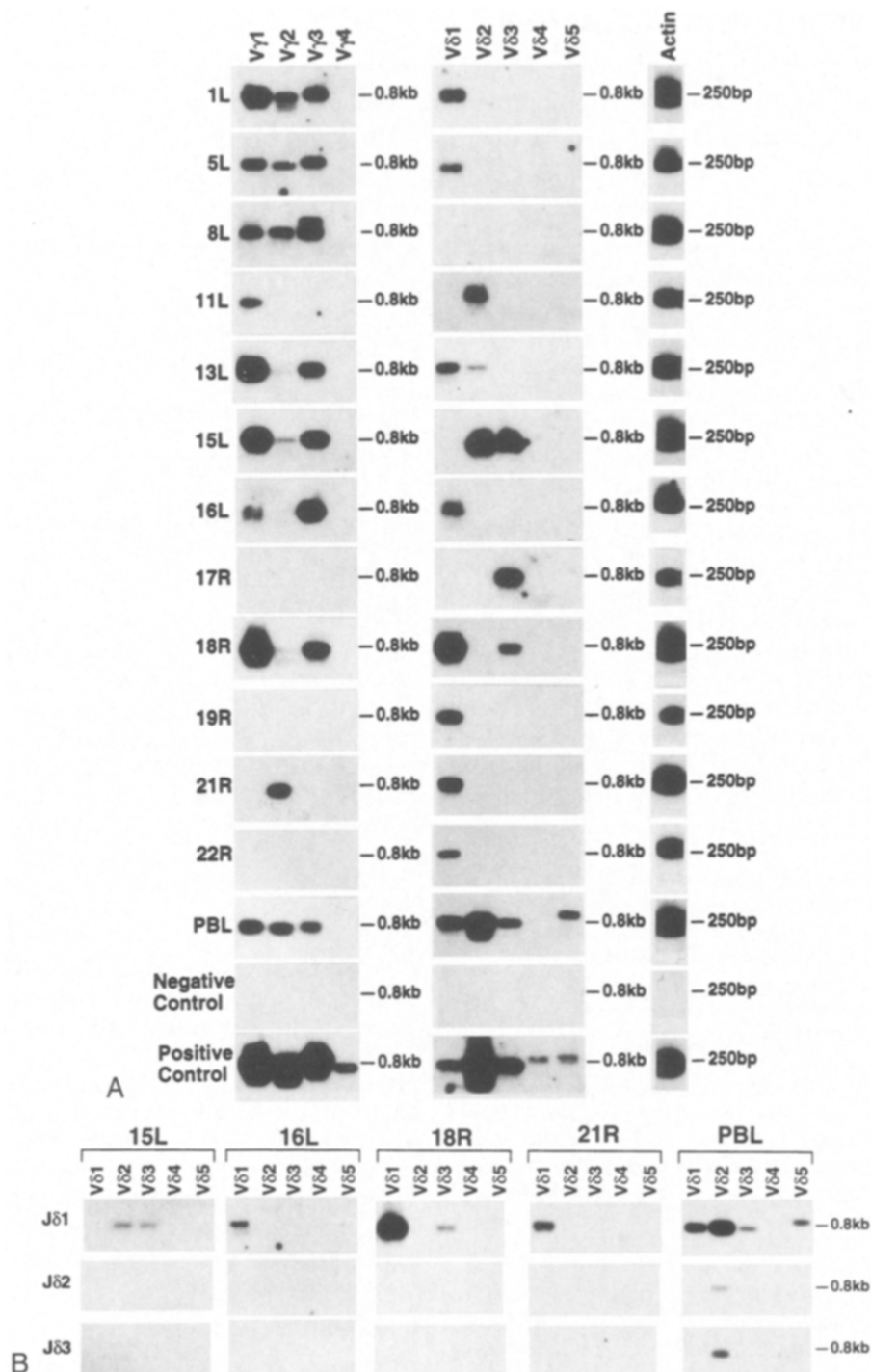


FIG. 1. A, Southern blot analysis of TCR γ and δ chain repertoire in human nasal mucosa of 12 individuals. TCR γ chains were amplified from normal nasal mucosal cDNA by using primers specific for V γ 1 to V γ 4 gene segments in combination with a C γ primer. TCR δ chains were similarly amplified by using primers specific for V δ 1 to V δ 5 gene segments in combination with a C δ primer. Southern blots were hybridized to an internal phosphorous 32-labeled C γ or C δ oligonucleotide probe. From 1L to 22R, Normal nasal mucosa from 12 volunteers; PBL, adult peripheral blood leukocyte cDNA; Negative Control, no cDNA; Positive Control, nasal polyp cDNA containing all the transcripts. **B**, J δ gene usage of TCR δ chains in nasal mucosa from four individuals. Human nasal mucosal cDNA was amplified by using primers for V δ 1 to V δ 5 in combination with a C δ primer. Amplified products were separated on agarose gels and Southern blots hybridized to J δ 1, J δ 2, and J δ 3 probes. Adult PBL cDNA was used for comparison.

cells exhibit higher levels of adhesion to epithelial and parenchymal cell monolayers than $\alpha\beta$ T cells and that the V δ 1 T-cell subset was more adhesive than V δ 2 cells, probably because the expression of the extracellular matrix (fibronectin) receptor very late antigen-5 was increased in the V δ 1 T-cell subset. These findings may explain why increased numbers of V δ 1 T cells compared with V δ 2 T cells are found in the epithelial and connective tissues.

REFERENCES

1. Porcelli S, Brenner MB, Band H. Biology of the human $\gamma\delta$ T-cell receptor. *Immunol Rev* 1991;120:137-83.
2. Uyemura K, Deans RJ, Band H, Ohmen J, Panchamoorthy G, Morita CT, et al. Evidence for clonal selection of $\gamma\delta$ T cells in response to a human pathogen. *J Exp Med* 1991;174:683-92.
3. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;162:156-9.
4. Wucherphennig KW, Liao YJ, Prendergast M, Prendergast J, Hafler, Strominger JL. Human fetal liver $\gamma\delta$ T cells predominantly use unusual rearrangements of the T cell receptor δ and γ loci expressed on both CD4⁺CD8⁻ and CD4⁻CD8⁻ $\gamma\delta$ T cells. *J Exp Med* 1993;177:425-32.
5. Lundqvist C, Hammarström ML. T-cell receptor $\gamma\delta$ -expressing intra-epithelial lymphocytes are present in normal and chronically inflamed human gingiva. *Immunology* 1993;79:38-45.
6. Nakajima S, Roswit WT, Look DC, Holtzman MJ. A hierarchy for integrin expression and adhesiveness among T cell subsets that is linked to TCR gene usage and emphasizes V δ 1⁺ $\gamma\delta$ T cell adherence and tissue retention. *J Immunol* 1995;155:1117-31.