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T-Cell repertoire in the tonsils of patients with IgA nephropathy

Takahara, Miki; Kishibe, Kan ; Nozawa, Hayabusa ; Harabuchi, Yasuaki

Title: T cell repertoire in the tonsil of patients with IgA nephropathy

Running title: T cell repertoire in the IgAN patients' tonsils.

Authors: Miki Takahara, M.D., Kan Kishibe, M.D., Ph.D., Hayabusa Nozawa, M.D., Yasuaki Harabuchi, M.D., Ph.D.

Departments of Otolaryngology-Head and Neck Surgery, Asahikawa Medical College, Asahikawa, Japan.

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E-mail address for authors: Yasuaki Harabuchi. hyasu@asahikawa-med.ac.jp

Mailing address for correspondence and reprints:

Yasuaki Harabuchi, Department of Otolaryngology-Head and Neck Surgery, Asahikawa Medical College, Midorigaoka E 2-1-1-1, Asahikawa, Hokkaido 078-8510, Japan.

Phone: +81-166-68-2554, Fax: +81-166-68-2559

Abstracts

Miki Takahara, Kan Kishibe, Hayabusa Nozawa, Yasuaki Harabuchi.

T cell repertoire in the tonsil of patients with IgA nephropathy. Acta Otolaryngol.

Objectives

It is known that IgA nephropathy (IgAN) often progresses to end-stage renal failure within a period of 20 years. There are many reports suggesting the relationship between the tonsillar autoimmune response and the pathogenesis of IgA nephropathy, however, definitive evidence was not detected. Recently, studies of T-cell receptor variable (TCR V) region gene usage in human autoimmune disease showed that the TCR V beta repertoire of pathogenic T-cells was highly restricted. In this study, we examined expression of TCR V beta families in tonsils from IgAN patients.

Method

The study group consisted of 7 IgAN patients and 7 obstructive sleep apnea syndrome (OSAS) patients. Total RNA was extracted from the tonsils of each patients, and expression of each TCR V beta subfamily gene were examined by reverse transcription-polymerase chain reaction (RT-PCR) method.

Result

V beta 6 is more frequently used in tonsils of IgAN patients than in those of OSAS patients

(p=0.01), and V beta 20 is more frequently used in OSAS patients than IgAN patients (p=0.01).

Conclusion

It was already reported that the frequency of V beta 6 subfamily usage is elevated in both peripheral blood lymphocytes (PBL) and renal tissues of IgAN patients. Therefore, our results suggested that tonsil may be one of the source for specific T-cells related to the pathogenesis of IgAN.

Key words : tonsillar focal infection, TCR V beta repertoire, RT-PCR

1. INTRODUCTION

World-wide, IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis.(1) IgA nephropathy was initially considered benign, but now it is recognized that progress to end-stage renal failure will often occur in these patients over a period of 20 years.(2) There are many reports suggesting the relationship between the tonsillar autoimmune response and the pathogenesis of IgA nephropathy from both clinical (3, 4) and experimental (5, 6) aspect. However, definitive evidence was not detected.

Recently, the determination of T-cell receptor variable region of beta gene (TCR V beta) usage on molecular basis of self-reactivity or super-antigen in human autoimmune disease receives attention. Initial studies of T-cell infiltration of the affected tissues in several autoimmune diseases such as Systemic Lupus Erythematosus (7) and Rheumatoid Arthritis (8) showed that the TCR v beta repertoire of pathogenic T-cell was highly restricted. In IgAN, TCR v beta 8 was commonly used in IgA nephropathy kidney biopsies.(9)

In this study, we investigated expression of TCR V beta families in IgAN tonsils by reverse transcription-polymerase chain reaction (RT-PCR), and tried to find the family commonly detected in these tonsils.

2. METHODS.

The study group consisted of 7 IgAN patients (19-42 years of age, mean 30 yers ; 2 males and 5 females) and 7 obstructive sleep apnea syndrome (OSAS) patients (31-49 years of age, mean 34 yers ; 5 males and 2 females) who had tonsillectomy in Asahikawa Medical Collage. Total RNAs were extracted from tonsil tissues of these patients by Acid Guanidinium Phenol Chloroform (AGPC) method with Sepasol-RNA1 (NACALAI TESQUE, Tokyo, Japan), and purified through treatment of DNaseI using message clean kit (GenHunter Corporation, Nashville, USA). cDNAs were synthesized from purified RNAs by MMLV reverse transcriptase (GenHunter Corporation). The primer sequences are shown in Table 1. These primer sequences were partially modified from the original sequences described in Choi's study.(10) Amplification (30 cycle, 56°C annealing temperature) was performed with cDNAs, Tag polymerase, and each different twenty V region and common C region primers. PCR products were loaded in 1% agarose gels and expression of each V-region gene subfamilies was semi-quantified by measurement of intensity for each amplified cDNA band on NIH image software. The percentage of each subfamily expression was calculated by dividing each subfamily expression by total expression of twenty subfamilies.

3. RESULTS

The percentages of expressions for twenty V beta subfamily genes in all patients were summarized in Figure 1a. In tonsils from all patients, V beta 13 was most commonly used among twenty V beta subfamilies, and the percentage of this gene ranged from 2.9% to 15.8% with a median of 6.9%. On the other hand, V beta 1 was most rarely used among them, and this percentage ranged from 0.2% to 3.2% with a median of 0.5%.

The usage of V beta gene subfamilies according to disease groups was shown in figure 1b. V beta 6 is more frequently used in tonsils of IgAN patients than in those of OSAS patients ($p=0.01$), and V beta 20 is more frequently used in OSAS patients than IgAN patients ($p=0.01$). In other V beta subfamilies, including V beta 8, there was not significant difference of percentages between tonsils from IgAN and from OSAS patients.

4. DISCUSSION

TCR V beta repertoire has been examined in several autoimmune diseases, (7, 8) because this analysis may lead to specific immunotherapeutic interventions for the treatment of these diseases.(11) Already Wu et al.(9) reported that TCR V beta 8 is commonly used in renal biopsy samples from IgAN patients compared to peripheral blood lymphocytes (PBL) from same patients. In our study, TCR V beta 8 was frequently used in tonsils, however, this was not

IgAN-specific subfamily. Moreover, our result suggested that TCR V beta 6 is IgAN-specific subfamily in tonsils. Wu et al.(9) also examined other TCR V beta subfamilies, and reported that usage of TCR V beta 6 is most frequent not only in renal samples but also in PBL from IgA patients. Geursen et al.(12) studied TCR V beta repertoire in PBL from 70 healthy donors and showed that Vbeta 6 is one of the commonly used subfamily, but not distinctly frequent. Therefore, we consider that the elevation of V beta 6 gene usage in PBL from IgA patients is also related to pathogenesis of IgAN. Based on these results including in our findings, in IgA patients, V beta 6 positive T-cells were activated by autoimmune response in tonsil, flow in blood, and may act as a trigger of IgAN in renal glomerulus.

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Corresponding author: Yasuaki Harabuchi.

Address of corresponding author:

Department of Otolaryngology-Head and Neck Surgery, Asahikawa Medical College,

Midorigaoka E 2-1-1-1, Asahikawa, Hokkaido 078-8510, Japan.

Phone: +81-166-68-2554, Fax: +81-166-68-2559

E-mail address: hyasu@asahikawa-med.ac.jp

Figure legend

Figure 1

a) Each TCR V beta gene subfamily expressions in all patients

By semiquantitative analysis of each TCR V beta gene subfamily expressions, V beta 13 was most commonly used among 20 subfamilies in tonsils from all patients. On the other hand, V beta 1 was least frequently used among 20 subfamilies.

b) Comparison between TCR V beta gene subfamily expressions in tonsils from IgAN patients and those from OSAS patients.

By the expression comparison between two disease groups, frequency in the use of V beta 6 was significantly higher in tonsils from IgAN patients than in those of OSAS patients. ($p=0.01$) On the other hand, V beta 20 is less frequently used in tonsils from IgAN patients than in those of OSAS patients. In usage of other subfamilies including in V beta 8, significant difference was not found between the two groups.

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Table 1

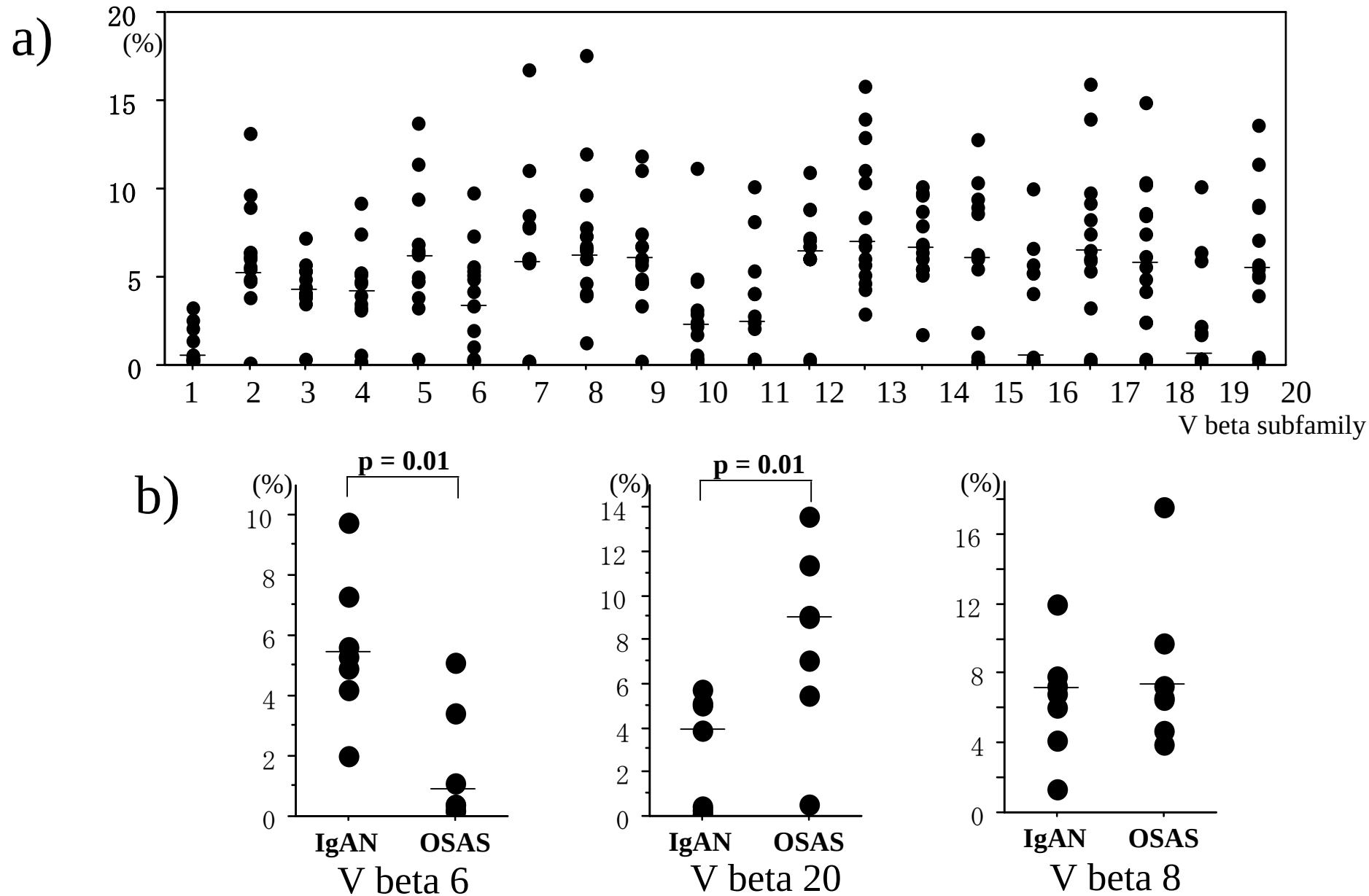
Each TCR V beta forword primer seqences

TCR V BETA1	CAAAAGGAAACATTCTTGAA	TCR V BETA11	AAGGGAGATCTTTCCTCTGA
TCR V BETA2	CCACATACGAGCAAGGCGTC	TCR V BETA12	CAAAGGAGAAGTCTCAGATGG
TCR V BETA3	TTCCTGAGGGGTACAGTGTC	TCR V BETA13	ATGGCTACAATGTCTCCAGA
TCR V BETA4	CATATGAGAGTGGATTTGTC	TCR V BETA14	AAGGGAGATGTTCCTGAAGG
TCR V BETA5	CAAAGGAAACTTCCCTGGTCG	TCR V BETA15	CAAAGGAGAGATCTCTGATGG
TCR V BETA6	ACGGGTGCGGCAGATGACTC	TCR V BETA16	TGGTATCGACGTGTTATGGG
TCR V BETA7	CAGTGTGCCAAGTCGCTTCTC	TCR V BETA17	GGAGATATAGCTGAAGGGTA
TCR V BETA8	ATAGATGATTCAGGGATGCC	TCR V BETA18	GGAATGCCAAAGGAACGATT
TCR V BETA9	TGAAACAGTTCCAAATCGCT	TCR V BETA19	GAGATGCACAAGAAGCGATT
TCR V BETA10	AAAGCAGAAATAATCAATGA	TCR V BETA20	GGCCTCCAGCTGCTCTTCTA

Common TCR C beta reverse primer seqence

TCR C BETA	TTCTGATGGCTCAAACA

Figure 1


a)
20
(%)
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
V beta subfamily
b)
p = 0.01
(%)
10
8
6
4
2
0
IgAN
OSAS
V beta 6
p = 0.01
(%)
14
12
10
8
6
4
2
0
IgAN
OSAS
V beta 20
(%)
16
12
8
4
0
IgAN
OSAS
V beta 8