Analysis of Accumulating Clonotypes of T Cell in Joints of a Spontaneous Murine Model of Rheumatoid Arthritis

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SKG mouse, as a model of spontaneous rheumatoid arthritis (RA) bred recent years, is similar to the patients with RA. We analyzed the clonotypes of T cell infiltrating into joints of SKG mice in initial stage and late stage of RA by using reverse transcriptase-polymerase chain reaction (RT-PCR) and subsequent single-strand conformation polymorphism (SSCP). The results indicated that the percentages of clonotypes TCR Vβ2 and Vβ8.2 of T cell clonotypes increased obviously to 72.3% and 60.2%, respectively. Mice number with identical TCR Vβ2 and Vβ8.2 clonotypes also clearly increased in late stage of disease to 100% and 75%, respectively. These results mean that T cells with TCR Vβ2 and Vβ8.2 clonotypes probably play an important role in RA progression of SKG mouse. Sequencing of the extracted DNA verified that common bands on SSCP gel were derived from the same T cell clones among samples from different joints. The results we obtained implied that RT-PCR/SSCP method was a sensitive and credible method for analyzing T cell clonotypes. When the T cells of SKG mouse were adoptively transferred to a nude mouse, it was verified that the T cells infiltrated in joints were related to morbid formation of RA. *Cellular & Molecular Immunology*. 2004;1(4):300-303.

Key Words: TCR, T cell clonotype, rheumatoid arthritis, RT-PCR, SSCP

Introduction

Rheumatoid Arthritis (RA) is a chronic inflammatory autoimmune disease characterized by destructive multiple arthritis (1). T cells have been proposed to play an important role in the disease initiation and progression (2, 3). Major pathological changes of RA are located in joints, where T cells infiltrated (4, 5). Therefore, analysis of T cell clonotypes accumulated in joints is vital for exploring the influence of T cells upon the morbid form of RA.

Sakaguchi made a murine model of spontaneous RA called as SKG mouse (Sakaguchi mouse) (6), which was bred by repeated hybridization of brothers and sisters of BALB/c mice with swollen joints. It was reported that RA of SKG mouse was similar to human RA. It is a fine animal model for the study of RA. On the basis of this, we selected SKG mouse to investigate T cell clonality during the disease course.

For studying the relationship between T cells and the disease *in vivo*, Yamamoto established RT-PCR/SSCP method for measurement of T cell clonotypes, that is the combination of reverse transcriptase-polymerase chain reaction (RT-PCR) with subfamily-specific TCR Vβ primers and single-strand conformation of polymorphism (SSCP) (7, 8), according to the different V regions of T cell receptor (TCR) (9). This method has been used to analyze T cell clonotypes of many animal models and human diseases (10-14). This investigation analyzed the clonality of T cells accumulated in joints of SKG mice.

Materials and Methods

Mice

SKG mice (male and female) were purchased from Tokyo Metropolian Institute of Gerontology, which were maintained under specific pathogen-free conditions in the animal facility of the University of Tokyo, School of Medicine.

RT-PCR/SSCP

Samples were prepared from joint tissues of SKG mice. The RT-PCR/SSCP analysis was performed roughly as previously described (11). Total RNA was converted to first-strand cDNA with reverse transcriptase and random hexamer oligonucleotides (100 pmol, BRL) at 42˚C for 2-3 h. The cDNA was mixed with each primer set (a Vβ sense primer and a Cβ anti-sense primer), according to the previously described sequences (15). PCR was performed with dNTP and Taq DNA polymerase (Promega, Co. Madison, WI) for 35 cycles (94˚C for 1.5 min, 54˚C for 2 min, 72˚C for 3 min). The amplified DNA was diluted with a denaturing solution (95% formamide, 10 mM EDTA, 0.1% bromophenol blue, 0.1% xylencynol) and for heat

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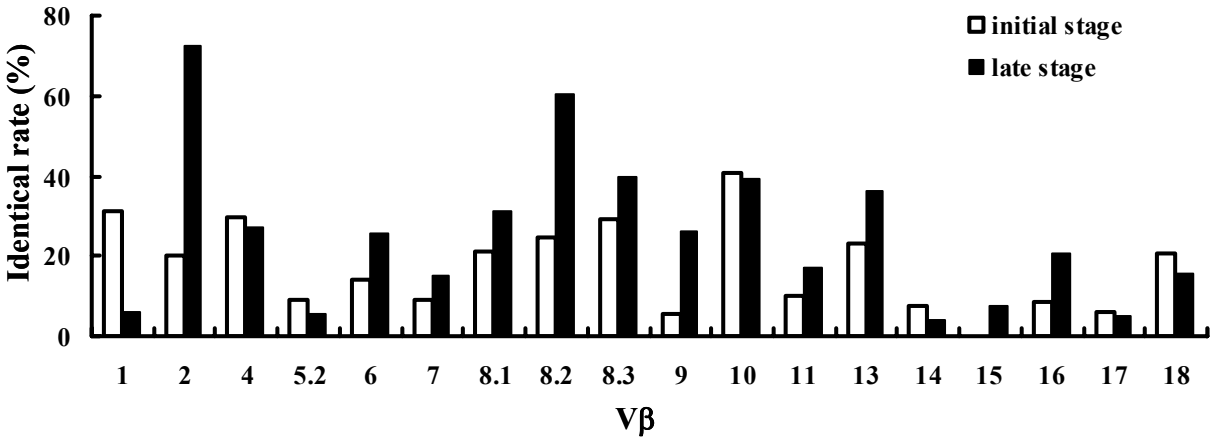

Figure 1. Identical clonotypes comparison of T cells accumulated in four joints of SKG mice at initial stage with late stage of RA.

denatured at 90˚C for 2 min and electrophoresed on non-denaturing 4% polyacrylamide gels containing 10% glycerol. After electrophoresis, the DNA was transferred onto Immobilon-S (Millopore Intertech, Bedford, MA) and then hybridized with a biotinylated internal common Cβ oligonucleotide probe: 5'-A(A, C)A A(G, C)G TGT TCC CAC CCG AGG TCG CTG TGT T-3'. The DNA was then visualized with streptoavidin, biotinlated alkaline phosphatase and a chemiluminescent substrate system (NE Blot Phototype detection kit, New England Biolabs, Berely, MA). The number of T cell clonotypes accumulated in joints was determined through clear bands detected from the background smear.

DNA sequencing

For DNA sequencing of PCR products on SSCP gel, a small area of the dried SSCP gel corresponding to the position of bands was cut out. The gel piece was immersed in 50 μl of TE.1 (10 mM Tris-HCL, 0.1 mM EDTA) in a micro-centrifuge tube and heated at 80˚C for 20 min. The extracted DNA was amplified by PCR with Vβ and Cβ primers, and cloned into a plasmid (pCR 2.1, Inritrogan, USA). The extracted DNA was determined by a cycle sequencing method using an automated DNA sequencer (373A Applied Biosystems, Foster City, CA).

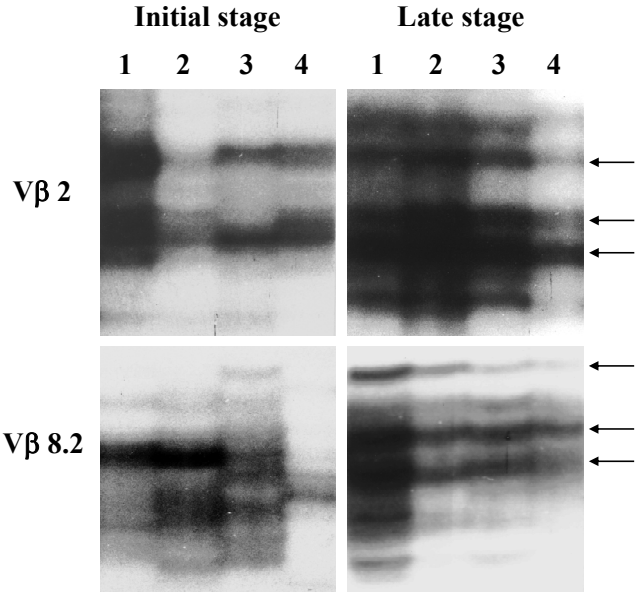

Figure 2. Accumulation of T cell clonotypes in four-foot joints of SKG mice in initial and late stages of RA analyzed by SSCP. Lane 1, right front foot; lane 2, left front foot; lane 3, right rear foot; lane 4, left rear foot.

T cell transformation

For investigating the pathogenicity of T cell clonotypes accumulated in joints, spleens were removed aseptically from SKG mice. Single-cell suspensions were prepared by teasing apart the spleens in HBSS and pushing them through in metal sieve with a syringe barrel. After washing with HBSS, splenic cells (1×10^6 cells/ml) were cultured in RPMI-1640 medium supplemented with 10% FCS in the presence of concanavalin A (ConA 10μg/ml, Sigma Chemical Co., St. Louis, MO). Three days later, a nude mouse was injected by tail vein with 0.5 ml and abdominal cavity with 0.5ml of the cells (5×10^7 cells/ml) stimulated with ConA. After the nude mouse showed the symptoms of arthritis, analysis and comparison of T cell clonality accumulated in SKG murine joints with that of nude mouse were made by RP-PCR/SSCP method.

Results

Comparison of rate of identical T cell clonotypes in joints of SKG mice

To investigate identical T cell clonotypes of foot joints of SKG mice in initial and late stages of RA, 3 months SKG mice were study targets of the disease in initial stage and 11 months SKG mice for late stage. The T cell clonotypes accumulated in four foot joints were compared in 3 months and 11 months mice (Figure 1).

The results showed that the identical rates of Vβ2 and Vβ8.2 clonotypes were 72.3% and 60.2% respectively in late stage of disease, and identical rates increased remarkably in late stage compared to that in initial stage. Comparison

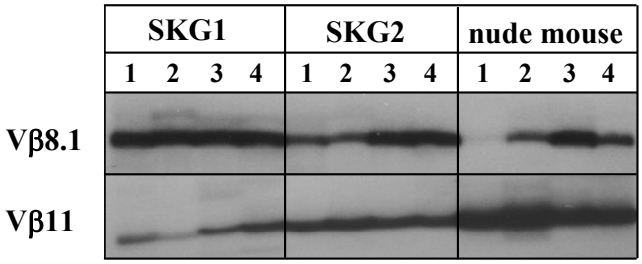

Figure 3. Identical T cell clonotypes in foot joints of SKG mice and a nude mouse. Lane 1, right front foot; lane 2, left front foot; lane 3, right rear foot; lane 4, left rear foot.

of T cell clonotypes in the four feet at the early and late of arthritis was showed in Figure 2.

In addition, the number of mice with identical Vβ2 and Vβ8.2 clonotypes was as high as 100% and 75% respectively. Although only two mice with identical Vβ8.2 clonotypes were in initial stage, all four mice with identical clonotypes were in late stage.

Sequence analysis of common clonotypes of murine four foot joints on SSCP gel

To verify if these common DNA bands on SSCP gel represent the same TCRβ chain sequence, DNA sequence was carried out. Four Common Vβ bands on SSCP gel from four foot joints of 11 months SKG mice were determined by DNA sequence. This result showed that DNA sequence of four bands was identical. It suggested that the bands with the same mobility were derived from the same T cell clones.

Analysis of the relationship between common T cell clonotypes and the disease progression

In order to investigate the pathological significance of the common clonotypes in four feet, we conducted an adoptive transfer experiment. The splencytes derived from two 4 months SKG mice were cultured for 3 days and were supplemented with ConA, then 1 ml of cells (5×10^7 cells/ml) were transferred into a nude mouse. Once the nude mouse showed swelling, a clonotypes analysis was made. This result revealed that there were some common clones in four foot joints of SKG mice and the nude mouse (for example Vβ8.1 and Vβ11, Figure 3).

Discussion

It has been demonstrated that T cells expand clonally in the synovium as well as the synovial fluid of RA patients (16-20). RA animal model similar with human RA is an important means for study of pathogenesis and pathological change of RA. SKG mouse is an animal model of spontaneous RA. There was a symmetrical swelling in both side joints and a chronic progression in two months SKG mouse. $CD4^+$ T cell infiltration was found under synovial membrane by immuno-staining. Pathological changes besides joints were interstitial pneumonia, lymph nodules and dermatitis. There was joint rigidity in many SKG mice over 6 months old. RA of the SKG mouse is similar to human RA. We consider that it is fit for studying T cell actions in initial and late stage of RA.

The method of RT-PCR/SSCP can be used to analyze T cell clonotypes even certain antigen is unknown. So it is very useful for the research on T cell clonotypes infiltrated into joints because the antigen caused it has not been found out. In this study, DNA sequence proved that common bands with same mobility on SSCP gel were identical T cell clones. In order to learn if the identical T cell clones accumulated in four foot joints of SKG mice were the same clones in four foot joints of nude mice, we chose Vβ8.1 and Vβ11 in four foot joints of SKG mice, which were clear bands in SSCP, to make an analysis with the samples of nude mice. T cell transformation showed that T cell clonotypes accumulated in joints of two kinds of mice were identical. It implied the T cell clones were correlated with RA. The identical clones might recognize certain common antigens that are involved in the pathogenesis of RA. These results suggested RT-PCR/SSCP method was sensitive and reliable method for investigating T cell clonotypes *in vivo* and *in vitro*.

This study showed that identical rate of Vβ2 and Vβ 8.2 clonotypes was higher in the late stage than in early stage of the disease, and the mice number with identical Vβ2 and Vβ8.2 clonotypes were also high. For this reason, it was considered that Vβ2 and Vβ8.2 clonotypes might play an important role in occurrence and development of RA.

The research indicated that many of the clones were common to all four foot joints of RA mice in the late stage of the disease, which was coincident with the result of Yukage Kobari. They found that accumulation of T cell clonality among joints in the early stage would become clonal restricted along with the disease progression (21).

Taken together, analysis of T cell clonotypes in the disease can not only learn the relationship between T cells and the disease but also imply that T cell clonotypes could be used as a treatment target.

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