Increased levels of serum IgM antibody to staphylococcal enterotoxin B in patients with rheumatoid arthritis

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Abstract

Objective—To investigate the role of superantigen in rheumatoid arthritis (RA) by assaying the serum levels of staphylococcal enterotoxin B (SEB) antibodies.

Methods—Serum IgG and IgM SEB antibodies were measured using an enzyme linked immunosorbent assay (ELISA), and confirmed by Western blot analysis. The T cell receptor V β (TCR V β) repertoire was analysed using the reverse transcriptase polymerase chain reaction.

Results—RA patients had increased levels of serum IgM SEB antibody compared with normal subjects, patients with systemic lupus erythematosus, Sjögren's syndrome, and Behçet's disease. The titres of rheumatoid factor (RF) showed no correlation with the levels of IgM SEB antibodies, and the levels of SEB antibodies were not inhibited by the addition of human immunoglobulin, or after absorption of RF. RA patients whose disease duration was less than 10 years had greater levels of serum IgM SEB antibodies than those with disease duration more than 10 years. The levels of IgM and IgG SEB antibodies in synovial fluid from RA patients were correlated with those in their sera. Western blot analysis detected IgM and IgG SEB antibodies as a band of approximately 30 kDa molecular size. The percentage of TCR V β 2, V β 5.2, and V β 12 in phytohaemagglutinin stimulated peripheral T cells correlated significantly with the levels of serum IgM SEB antibody in RA patients.

Conclusion—These results suggest that SEB, one of the superantigens, may have a critical role in the pathogenesis of RA.

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Rheumatoid arthritis (RA) is an autoimmune disease characterised by marked inflammation and destruction of several joints. Histological studies of RA synovium show proliferation of synovial cells and infiltration of mononuclear cells in association with destruction of cartilage and bone.¹ Bacterial infection may be one of the triggering events for autoimmune disease in a genetically susceptible patient. Several micro-organisms elaborate substances or toxins that suppress or stimulate immune cells, and it is conceivable that such products

modulate the host defense against the micro-organisms and thus have a role in the immune pathogenesis of autoimmune disease. Several micro-organisms are known to cause chronic diseases resembling RA in mice and humans.^{2–5}

Bacterial exotoxins are exogenous superantigens which activate a wide range of T cells bearing particular V β receptor families. Another type of superantigens are the Mls antigens in mice that have been identified as the mouse retroviral gene product.^{6–12} *Staphylococcus aureus* produces many exogenous superantigens such as SEA, SEB, SEC1, SEC2, SEC3, SED, SEE, and TSST-1. Of these, SEB stimulates synthesis of an antibody which is detectable in the sera not only of staphylococcus infected patients, but also of healthy subjects.^{13–14}

A possible role of superantigen in the autoimmune response has been reported. T cells which have particular T cell receptor (TCR) V β families proliferate in affected joints, implicating superantigen in the pathogenesis of RA.^{15–16} However, reports have shown expanded T cells which express a variety of T cell receptor V β repertoires in affected joints and the role of superantigens in RA thus remains controversial.^{15–22}

In the present study, we evaluated the humoral role of superantigens in RA by assaying the serum levels of SEB antibodies.

Patients and methods

PATIENTS

Thirty four patients (five men, 29 women) who fulfilled the American Rheumatism Association (ARA) criteria²³ for definite or classical RA were the subjects of the present study and comprised the RA group. Their mean age was 56.9 (SD 13.5) years (range 21–78). Sera and synovial fluid samples were obtained simultaneously from 13 of the patients in this group. Sera from 30 patients who fulfilled the ARA criteria²⁴ for systemic lupus erythematosus (SLE) were also studied; the mean age of this group was 41.0 (14.7) years (range 21–73). Sera from 16 patients with Sjögren's syndrome who fulfilled the criteria proposed by the European Community Study Group for Sjögren's syndrome,²⁵ and six patients with Behçet's disease who fulfilled the criteria established by the international study group for Behçet's disease²⁶ were also investigated. The mean ages of these two groups were 55.3 (9.7)

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and 46.7 (8.7) years, respectively. All patients were outpatients in the Clinical Division of Rheumatology, the First Department of Internal Medicine, Nagasaki University School of Medicine.

At the time of study, all patients with RA were taking a non-steroidal anti-inflammatory drug and 22 patients were administered 2.5–15 mg of prednisolone daily. The activity indices, including joint score and duration of morning stiffness, were determined by two rheumatologists in our department. Joint score was defined as the sum of the painful joint score and swollen joint score, each graded 0–3+.²⁷ Functional classes and clinical stages were determined according to Steinbrocker *et al.*²⁸ Agglutination of red blood cells coated with rabbit IgG (RAHA) and enzyme linked immunosorbent assay (ELISA) of cells pre-coated with human IgG were used to determine the presence of serum rheumatoid factor (RF). When at least one of the above assays was positive at the time that serum SEB antibody was determined, the patient was judged RF positive (table).

A control group comprised 22 gender and age matched healthy normal subjects (53 (10.5) years, three men and 19 women).

DETECTION OF ANTIBODIES BY ELISA

Antibodies to SEB and SEA (Sigma Chemical, St Louis, MA) were detected by ELISA. In brief, each well of polystyrene microtitre plates (Falcon 3915, Becton Dickinson, Lincoln Park, NJ) was filled with 100 µg/ml of SEB or SEA in phosphate buffered saline (PBS) solution and incubated overnight at 4°C. Plates were washed three times with PBS containing

0.05% Tween 20, and drained. Each well was filled with 150 µl of 3% bovine serum albumin (BSA) in PBS with 0.02% sodium azide for two hours at 4°C. Fifty microlitres of 1:100 diluted samples of sera or synovial fluid was placed into each well and the plates shaken gently for one hour at room temperature. After thorough washing, each well was rinsed repeatedly and filled with 50 µl of 1:500 diluted, peroxidase labelled antihuman IgM or antihuman IgG antibody (Cappel, Durham, NC) for one hour at 4°C. Finally, bound antibodies were visualised with O-phenylenediamine (30 minutes at room temperature). The absorbance was measured at 450 nm using an Immuno Reader NJ-2001 (Japan Inter Med, Tokyo, Japan). The amount of SEB antibody was calculated as follows:

Amount of antibody (%) =

$$\frac{\text{OD}_{450} \text{ of sample serum} - \text{OD}_{450} \text{ of PBS}}{\text{OD}_{450} \text{ of positive control serum} - \text{OD}_{450} \text{ of PBS}} \times 100$$

ABSORPTION OF RHEUMATOID FACTOR FROM SERUM

Cyanogen bromide activated Sepharose 4B (CNBr activated Sepharose 4B, Pharmacia, Uppsala, Sweden) was coupled to human immunoglobulin (Midorijui, Osaka, Japan) as follows: 5 ml of human immunoglobulin (10 mg/ml) in 0.5 mol/l sodium phosphate (pH 7.5) was mixed with CNBr activated beads overnight at room temperature. The beads were washed twice with sodium phosphate and once with 1 mol/l sodium chloride, 0.05 mol/l sodium phosphate (pH 7.5), then incubated with 10 volumes of 100 mmol/l ethanolamine (pH 7.5) for four hours. They were again

Clinical profile of 34 rheumatoid arthritis patients

Patient No	Age (yr)/sex	Stage	Class	Disease duration (yr)	Tender and swelling joints (No)	Duration of morning stiffness (min)	Level of IgM SEB antibody (%)	RF	ESR (mm/1st h)	Medications
1	56/F	1	1	1	4	10	100	–	8	NSAID
2	59/F	1	1	3	5	1	40	+	33	NSAID
3	58/F	2	2	4	3	0	51	+	88	NSAID, SASP
4	48/F	2	2	4	20	1	44	+	26	PSL, NSAID, SASP
5	64/F	2	2	4	28	30	38	+	73	PSL, NSAID, SASP
6	25/F	2	2	5	12	30	76	+	51	NSAID, Bu
7	52/M	2	2	6	6	30	91	+	89	NSAID, Bu
8	74/F	1	2	6	6	0	75	–	47	D-pen
9	57/F	3	2	7	24	0	63	+	69	PSL, NSAID, Bu
10	68/F	4	2	7	1	0	27	+	83	NSAID, Auranofin
11	60/M	4	4	7	29	300	45	+	16	PSL, NSAID, SASP
12	68/F	3	2	8	6	0	30	+	77	PSL, NSAID, MTX
13	79/F	2	2	10	1	0	36	+	30	PSL, NSAID,
14	52/F	3	2	29	30	90	37	–	9	PSL, NSAID, Bu, MTX
15	30/F	4	2	10	8	180	23	–	22	PSL
16	60/F	3	3	12	6	60	13	+	40	PSL, NSAID, MTX
17	60/F	4	4	12	10	60	10	+	140	PSL, NSAID
18	38/F	4	2	13	10	0	10	–	101	PSL, NSAID
19	67/F	4	2	14	2	20	58	+	44	PSL, NSAID, MTX
20	66/F	4	3	23	1	0	36	+	28	PSL, NSAID, MTX
21	48/F	4	2	6	11	0	2	+	42	PSL
22	50/F	4	2	16	18	60	19	+	23	PSL, NSAID, SASP
23	38/F	3	2	18	13	20	45	+	23	PSL, NSAID, D-pen
24	21/F	2	2	2	12	0	10	+	15	PSL, NSAID
25	59/F	4	3	23	1	0	3	+	52	NSAID
26	63/F	2	2	6	10	60	–3	–	23	NSAID
27	68/F	4	3	25	15	60	–3	–	33	PSL, NSAID, Bu
28	59/F	3	2	34	6	30	20	+	35	NSAID, SASP
29	51/M	4	3	37	44	>480	3	+	132	PSL, NSAID, SASP
30	67/F	2	1	38	1	0	26	–	75	NSAID
31	60/F	4	3	38	0	0	72	+	98	NSAID
32	70/F	4	2	48	3	0	22	+	85	PSL, NSAID
33	73/F	4	3	51	8	0	39	+	18	PSL, NSAID
34	75/F	4	2	58	19	30	23	+	73	PSL, NSAID, MTX

RF = Rheumatoid factor; ESR = erythrocyte sedimentation rate; PSL = prednisolone; NSAID = non-steroidal anti-inflammatory drug; MTX = methotrexate; SASP = sulphasalazine; Bu = buccamine; D-pen = D-penicillamine.

washed (with PBS) and merthiolate (Sigma Chemical Co.) was added, to 0.01%.²⁹

Serum samples were incubated with the immunoglobulin coupled beads (1:2 vols) for two hours at room temperature. The levels of serum IgG and IgM SEB antibodies which had or had not been incubated with immunoglobulin coupled beads were assayed and compared.

WESTERN BLOT ANALYSIS OF SEB ANTIBODY

SEB was separated by electrophoresis on 12.5% sodium dodecyl sulphate (SDS) polyacrylamide slab gels under reducing conditions with 2-mercaptoethanol, then transferred electrophoretically to a nylon membrane (NEN Research Products, Boston, MA). Individual lanes were incubated with 50-fold diluted sera, then incubated with 500-fold diluted biotinylated goat antihuman IgG and antihuman IgM (Zymed, San Francisco, CA), followed by incubation with peroxidase labelled streptavidin (Zymed). Specific reactivity was evaluated by staining the samples with 4-chloro-1-naphthol (Gibco BRL, Gaithersburg, MD).

CELL CULTURE AND ANALYSIS OF TCR V β REPERTOIRE

Peripheral mononuclear cells were isolated from heparinised blood of RA patients and healthy subjects by Ficoll-Conray (Daiichi pharmaceutical, Tokyo, Japan) density gradient centrifugation. The cells were cultured with RPMI1640 medium containing 2 mmol/l L-glutamine, 50 U/ml penicillin, and 50 μ g/ml streptomycin supplemented with 10% heat inactivated fetal bovine serum (Gibco, Gaithersburg, MD), stimulated with 5.0 μ g/ml of phytohaemagglutinin (PHA) (Sigma) for three days and stored at -80°C until required for extraction of RNA.

Total RNA was prepared from PHA stimulated peripheral T cells according to Chomezynski *et al.*³⁰ Three micrograms of total RNA was used for the synthesis of single stranded cDNA using reverse transcriptase (BRL, Bethesda, ML) and oligo dT₁₄ primer. Five percent of each cDNA sample was amplified using a V β specific primer with a C β primer at a final concentration of 0.3 μ mol/l. Amplification was performed for 22 cycles with 2.0 units of Taq polymerase (Wako Chem, Tokyo) under the following conditions: 95°C melting, 55°C annealing, 72°C extension, for one minute each.

The amplified products separated by polyacrylamide gel electrophoresis (PAGE) were transferred onto nylon filters (Gene Screen Plus; DuPont, Boston, MA). Hybridisation was performed with oligonucleotide probes labelled with [³²P] γ -ATP (Amersham), and the membrane was exposed to an imaging plate (BAS-III; Fuji Photo Film, Tokyo, Japan). The radioactivity of specific bands was measured by the Bio-imaging Analyzer (BAS 2000; Fuji Photo Film). The TCR V β repertoire was calculated as follows:

Percentage of each TCR V β =

$$\frac{\text{radioactivity of specific band of each V}\beta \text{ family}}{\text{sum of radioactivities of total V}\beta \text{ families}} \times 100$$

RELATIONSHIP BETWEEN LEVEL OF IgM SEB ANTIBODY AND CLINICAL FEATURE

We studied the relationship between the level of IgM SEB antibody and clinical features including gender, age, clinical stage and class, duration of morning stiffness, number of tender and swelling joints, erythrocyte sedimentation rate (ESR), serum levels of RF and IgM.

STATISTICAL ANALYSIS

Student's *t* test, the paired *t* test, or the Wilcoxon test was used to compare the results among different groups. Values of *p* < 0.05 were considered statistically significant.

Results

IgM AND IgG ISOTYPE SEB ANTIBODIES IN SERA FROM RA PATIENTS

In preliminary experiments, one patient with RA (No 1 in the table) had markedly increased concentrations of IgM and IgG SEB antibodies. When the serum was diluted serially, the OD₄₅₀ of IgM class and IgG class SEB antibodies correlated with the log of serum dilution (data not shown). We used the serum from this patient as positive control serum throughout the following experiments.

The mean serum levels of IgM class SEB antibodies were 35.7 (SD 26.3)% in 34 patients with RA (fig 1), 2.8 (7.4)% in 30 patients with SLE, 7.8 (6.4)% in 16 patients with Sjögren's syndrome, 7.5 (9.7)% in six patients with Behçet's disease, and 5.9 (19.5)% in 22 normal subjects. The IgM SEB antibody levels in sera from RA patients were significantly greater than those in all the other groups (*p* < 0.05). There was no significant difference between serum levels of IgG SEB antibodies in RA patients and normal subjects (80.2 (24.5)% and 70.5 (22.2)%, respectively). The IgG SEB antibody levels in sera from SLE patients were smaller than those in RA patients, but the difference was not significant.

RELATIONSHIP BETWEEN LEVEL OF IgM SEB ANTIBODY AND CLINICAL FEATURES

The presence or absence of serum RF (assayed by ELISA) did not affect the level of IgM SEB antibody (RF positive group: 35.2 (23.4)%; RF negative group: 33.1 (34.4)%) (fig 2A). The level of IgM SEB antibody did not correlate with the titre of RAHA assayed by the passive haemagglutination method (fig 2B) or with the concentration of total IgM (fig 2C) in the sera. Furthermore, neither a positive nor a negative correlation was found between IgM SEB antibody levels and other clinical indices. However, there was a significant correlation between level of IgM SEB antibody and

Figure 1 IgM (left) and IgG (right) class SEB antibodies in sera from patients with rheumatoid arthritis (RA) ($n = 34$), systemic lupus erythematosus (SLE) ($n = 30$), Sjögren's syndrome (SS) ($n = 16$), Behçet's disease (BD) ($n = 6$) and healthy control subjects (C) ($n = 22$). Bars represent mean (SD) for each group. ** $p < 0.01$ compared with all other groups.

duration of disease. The patients were tentatively classified into two groups according to the duration of disease: serum concentrations of IgM class SEB antibodies in a group of recent onset RA patients (within 10 years) were significantly greater than those in a group of patients with a history of disease of more

than 10 years ($56.3 (22.8)\%$ v $18.4 (21.2)\%$, respectively ($p < 0.05$)) (fig 3A). In particular, there was a significant negative correlation between IgM class SEB antibody levels and duration of disease in 26 patients with RA within 30 years from onset ($r = -0.714$; $p < 0.01$) (fig 3B).

Figure 2 A: Mean (SD) serum IgM SEB antibody levels in rheumatoid factor (RF) positive and negative groups. B: Correlation between IgM SEB antibody levels and RAHA in RA patients. C: Correlation between the serum levels of IgM SEB antibody and serum concentration of total IgM.

DETECTION OF SEB ANTIBODIES IN SYNOVIAL FLUID FROM RA PATIENTS

Levels of IgG and IgM SEB antibodies in synovial fluids correlated well with those in the respective sera obtained simultaneously, in 13 patients with RA (IgM class: $r = 0.692$, $p < 0.05$; IgG class: $r = 0.914$, $p < 0.05$) (fig 4). The levels of IgG and IgM SEB antibodies in

Figure 3 IgM class SEB antibody levels and duration of RA. A: RA patients whose duration of disease was less than or more than 10 years. * $p < 0.05$ v RA patients whose disease duration was within 10 years. B: Duration of disease in RA patients and levels of IgM class SEB antibody calculated.

Figure 4 Detection of SEB antibodies in synovial fluid and paired sera from 13 patients with RA. A: IgM class antibodies ($r = 0.692$, $p < 0.05$); B: IgG class antibodies ($r = 0.914$, $p < 0.05$).

the synovial fluid were similar to those in the sera.

WESTERN BLOT ANALYSIS OF SEB ANTIBODIES

Western blot analysis revealed serum SEB antibody as a band at approximately 30 kDa, which is compatible with the molecular weight of SEB (fig 5).

Neither IgM nor IgG SEB antibody was detected by Western blot analysis of serum in which levels of IgG and IgM SEB antibodies were both low by ELISA (serum A). Only IgG SEB antibody was detected by Western blot analysis of serum in which the level of IgG SEB antibody in ELISA was high but IgM SEB antibody was low by ELISA (serum B). Both IgG and IgM SEB antibodies were detected by Western blot analysis in sera in which levels of IgG and IgM SEB antibodies were both high by ELISA (sera C and D).

SEB antibodies on Western blot analysis were negative in serum A, which was RF positive, but were detected by this technique not only in sera B and C, which were RF positive, but also in serum D, which was RF negative (fig 5).

Figure 5 Western blot analysis of IgM and IgG SEB antibodies. LANES A (patient No 2 in the table): representative serum without IgM class and IgG class SEB antibodies (rheumatoid factor (RF) positive). LANES B (patient No 21 in table): representative serum containing IgG, but no IgM, class SEB antibodies (RF positive). LANES C (patient No 7 in table): representative serum containing both IgM and IgG class SEB antibodies (RF positive). LANES D (patient No 8 in table): representative serum containing both IgM and IgG class SEB antibodies (RF negative).

SERUM LEVELS OF SEA ANTIBODIES IN RA PATIENTS

Antibodies to SEA in sera from 19 randomly selected RA patients were present in small amounts compared with SEB antibodies (fig 6).

SERUM LEVELS OF IgG AND IgM SEB ANTIBODIES BLOCKED BY HUMAN IMMUNOGLOBULIN AND SEB, AND AFTER ABSORPTION OF RHEUMATOID FACTOR

Serum levels of IgG SEB antibody increased, but serum levels of IgM SEB antibody were not significantly changed, in the presence of human immunoglobulin 1 mg/ml (fig 7). Addition of SEB 100 μ g/ml to the serum samples significantly decreased the levels of both IgG and IgM SEB antibodies (fig 7). Absorption of RF by human immunoglobulin bound beads did not make a significant difference to the serum levels of IgG and IgM SEB antibodies (fig 7).

TCR $\text{V}\beta$ REPERTOIRE OF PERIPHERAL T CELLS STIMULATED WITH PHA

There was a significant correlation between percentages of $\text{V}\beta 2$, $\text{V}\beta 5.2$, $\text{V}\beta 11$, $\text{V}\beta 12$, $\text{V}\beta 14$, or $\text{V}\beta 16$ and level of IgM SEB antibodies in RA patients ($r = 0.949$, -0.967 , -0.865 , 0.932 , -0.860 , -0.821 , respectively) (fig 8A). The percentage of $\text{V}\beta 3$ or $\text{V}\beta 20$ was significantly greater in RA patients than in normal subjects, and the percentage of $\text{V}\beta 11$ was significantly smaller in RA patients than in normal subjects in PHA stimulated T cells (fig 8B).

Discussion

Serum antibodies to staphylococcal enterotoxins have been assayed previously in patients infected with *Staphylococcus aureus* and in healthy controls.^{13 14} These reports suggested that certain humans may be carriers of particular strains of *S aureus*. The titres of the enterotoxin antibodies in the sera of healthy persons were lower than those in recently infected patients.¹⁴ Thus a latent infection with *S aureus* may modulate the immune system in

Figure 6 Serum levels of IgM (A) and IgG (B) SEA antibody in 19 patients with RA, compared with SEB antibody from the same patients.

an individual through the effect of enterotoxins.

Our results demonstrated that the levels of IgM SEB antibodies in sera of RA patients were significantly greater than those of normal subjects. It seems unlikely that RF or non-specific binding of IgM affected our assay, because no correlation was observed between the levels of IgM SEB antibodies and the titre of RAHA or total IgM levels (fig 2). In addition, as shown in the table, sera from patients Nos 2, 10, 11, 16, 17, 20, 21, and 30 were negative for RF and their serum levels of IgM SEB antibody were 33.1 (34.4)%. There was no significant difference between the levels of IgM SEB antibody in seronegative and seropositive RA sera (35.2 (23.4)%). Furthermore, the levels of SEB antibodies were not reduced in the presence of human IgG which is the target of RF, or after the absorption of RF by human immunoglobulin bound beads (fig 7). The levels of SEB antibodies were decreased by the addition of SEB (fig 7), but not of SEA (data not shown). Although the purity of SEB used in the present assay may be questioned, we identified only one specific band at approximately 30 kDa on SDS-PAGE (data not shown), that was confirmed by Western blot analysis which also showed a specific band at approximately 30 kDa, both in

Figure 7 Serum IgG (□) and IgM (■) SEB antibodies in the presence (+) or absence (-) of human IgG (hIgG) 1 mg/ml or SEB 100 mg/ml, or with (+) or without (-) absorption of rheumatoid factor by human immunoglobulin bound beads. Y axis indicates OD value. ** and ††: Significant difference between matching pairs ($p < 0.01$).

RF positive and in RF negative sera. We could not find any correlation between the levels of serum SEB antibodies and those of serum SEA antibodies, indicating that increased levels of IgM SEB antibodies do not necessarily reflect the presence of antibody to SEA contaminating SEB from commercial sources.¹⁵ However, the levels of serum antibodies against SEC, SED, or SEE may be increased in RA patients; in particular, cross reactivity between SEB antibodies and SEC antibodies has been reported.¹⁶ The increase in serum SEB antibody in RA demonstrated in the present study may merely reflect that, in RA, an abnormal immune response to a superantigen caused the increased production of antibody to the antigen. The levels of IgM SEB antibody in RA were greater in the group with a disease duration of no more than 10 years, and decreased in proportion to the duration of RA. Such findings suggest that the increased levels of the antibodies may be related to the initiation of RA.

A role for superantigens has been proposed in previous reports which investigated T cell receptor V β involvement in affected joints. However, the specific increase in reported V β involvement was variable, so the role of superantigen in RA remains controversial.¹⁵⁻²² It is difficult to interpret the results obtained from the analyses of TCR V β repertoire as shown in figure 8. The statistically lower probability for the correlation between levels of IgM SEB antibody and percentage of the V β 2, V β 5.2, or V β 12 families in RA patients, and for the difference in V β 3 and V β 11 families between RA patients and normal subjects, may imply that these V β families are the significant T cell candidates for the increased levels of IgM SEB antibody in RA patients. Among these, V β 3 and V β 12 have previously been reported to be the families responsive to SEB.³¹ The SEB antibodies seem not to be produced in local lesions such as synovial tissues, because their levels in the synovial fluids were no greater than those in the sera. The synovial cells in the affected joints of RA overexpress class II MHC antigens on their surface, so exposure to superantigens may initiate or perpetuate localised inflammation by activating T cells with synovial cells. It is also possible that SEB serves as a coadhesion molecule which helps to activate T cells that recognise putative autoantigens in RA.^{17 18 32 33}

Although the precise mechanism for increased IgM SEB antibodies in RA sera remains unknown, the findings of the present study support the hypothesis that superantigens have a role in triggering or perpetuating autoimmune diseases.³⁴ With a perturbation of the immune system of this kind, autoreactive T cells specific for the putative antigens in RA joints may be activated.³⁵ There have been several reports which support a relationship between RA and *Staphylococcus aureus*: the *S aureus* strain Cowan 1 stimulates the production of IgM RF³⁶⁻³⁹ and SED is also reported to stimulate production of RF in vitro.⁴⁰ Preferential binding of SEB to DR4, the class II MHC haplotype susceptible to RA,

Figure 8 TCR V β repertoire analysed by semiquantitative reverse transcriptase polymerase chain reaction. A: TCR V β repertoire in six RA patients whose serum levels of IgM SEB antibody were 67% (●), 52% (▲), 48% (■), 26% (○), 15% (△), and 13% (□), respectively. Significant correlation with the levels of IgM SEB antibody: * $p < 0.05$; ** $p < 0.01$. B: Difference in peripheral TCR V β repertoire between patients with RA (○) and controls (●). Significant difference from value of respective TCR V β in normal controls: * $p < 0.05$; ** $p < 0.01$.

has been indicated.⁴¹ The present study demonstrated increased serum levels of only the IgM SEB antibody in RA. Further investigations are required to clarify the detailed mechanism of this increase.

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