Characterization of the T Cell Receptor Repertoire Causing Collagen Arthritis in Mice

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Summary

Collagen type II–induced arthritis (CIA) is generated in susceptible rodent strains by intradermal injections of homologous or heterologous native type II collagen in complete Freund's adjuvant. Symptoms of CIA are analogous to those of the human autoimmune disease, rheumatoid arthritis. CIA is a model system for T cell–mediated autoimmune disease. To study the T cell receptor (TCR) repertoire of bovine type II–specific T cells that may be involved in the pathogenesis of CIA in DBA/1Lac.J (H-2^q) mice, 13 clonally distinct T cell hybridomas specific for bovine type II collagen have been established and the α and β chains of their TCRs have been analyzed. These T cell hybridomas recognize epitopes that are shared by type II collagens from distinct species and not by type I collagens, and exhibit a highly restricted TCR-α/β repertoire. The α chains of the TCRs employ three Vα gene subfamilies (Vα11, Vα8, and Vα22) and four Jα gene segments (Jα42, Jα24, Jα37, and Jα32). The Vα22 is a newly identified subfamily consisting of approximately four to six members, and exhibits a high degree of polymorphism among four mouse strains of distinct Vα haplotypes. In addition, the β chains of the TCRs employ three Vβ gene subfamilies (Vβ8, Vβ1, and Vβ6), however the Vβ8.2 gene segment is preferentially utilized (58.3%). In contrast, the Jβ gene segment usage is more heterogeneous. On the basis of the highly limited TCR-α/β repertoire of the TCRs of the panel of bovine type II–specific T cell hybrid clones, a significant reduction (60%) of the incidence of arthritis in DBA/1Lac.J mice is accomplished by the use of anti-Vβ8.2 antibody therapy.

Collagen type II–induced arthritis (CIA)[1] in animals is an experimental animal model system of the human autoimmune disease, rheumatoid arthritis (1–3). CIA is induced in susceptible rodents by intradermal injections of homologous or heterologous native collagen type II (1, 2). In contrast, similar injections of other joint tissue proteins such as collagen type I and proteoglycans do not lead to arthritis. In addition, susceptibility to CIA in rodents is linked to MHC genes (4, 5). Among the inbred mouse strains, only mice of the H-2^q and H-2^r haplotypes generally acquire an inflammatory polyarthritis upon immunization with collagen type II in CFA (5). However, SWR (H-2^q) and RIII (H-2^r) inbred mouse strains are resistant to CIA (6, 7), suggesting that non-MHC genes are also crucial for the induction of the disease.

Previous studies indicate that CIA is associated with a high level of both cellular and humoral responses to collagen type II. However, the role of Abs and T lymphocytes in the pathogenesis of the disease is ill-defined. It has been reported that transfer of anti-collagen type II Abs to naive animals results in transient synovitis with a histopathologic picture different from that seen in CIA (8). Hence, antibodies to collagen type II alone are not sufficient for the development of the prototypical lesions associated with arthritis. In contrast, adoptive transfer of collagen type II Abs and CD4 T cells isolated from mice immunized with denatured collagen type II, can together promote the development of classical arthritis (9). This finding clearly demonstrates the synergistic effect between humoral and cellular responses in initiating polyarthritis. The important role of CD4 T cells in the induction of arthritis is manifested indirectly by the in vivo administration of anti-MHC class II Abs, resulting in the inhibition of CIA (10). Moreover, direct involvement of T cells has been illustrated by the resistance of athymic nude rats, and anti-CD4 treated mice to CIA (11, 12).

The analysis of TCRs of myelin basic protein (MBP)–reac-

[1] *Abbreviations used in this paper:* B.CII, bovine collagen type II; C.CII, chicken collagen type II; CIA, collagen type II–induced arthritis; EAE, experimental allergic encephalomyelitis; MBP, myelin basic protein; NOD, nonobese diabetic.

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tive T cells derived from the experimental allergic encephalomyelitis (EAE)-susceptible B10.PL mice revealed that the TCR-α/β repertoire is highly restricted, using only two Vα gene segments (Vα2.3 and Vα4.2) and two Vβ gene segments (Vβ8.2 and Vβ13) (13). In addition, a similar finding has been obtained from T cell clones derived from PL/J mice and Lewis rats (14, 15). Consequently, the prevention and reversal of EAE in B10.PL mice was accomplished using a combination of anti-Vβ8.2 and anti-Vβ13 mAbs (16). These surprising results have suggested that autoaggressive T cells in other autoimmune diseases may generally exhibit restricted TCR repertoires, and that anti-TCR therapies may prevent and/or cure autoimmune diseases. However, the notion that pathogenic T cells in autoimmune diseases exhibit limited TCR repertoires cannot be generalized to every autoimmune disease since molecular characterization of the TCRs used by islet-infiltrating T cells of nonobese diabetic (NOD) mice revealed that the usage of TCR V and J gene segments is not restricted as in the case of EAE (17, 18).

The question of whether or not the TCR repertoire of T cells involved in the induction of CIA is limited has been addressed indirectly by two different groups. The first group has found that DBA/1 mice treated with (*a*) anti-Vβ8.1,8.2 mAb (KJ16) and (*b*) anti-Vβ5.1,5.2 mAb (MR9.4), before injections of bovine collagen type II (B.CII) had a reduced incidence of arthritis, 28.6 and 50%, respectively, as opposed to an 84.6% incidence in control mice (19). This observation suggests that T cells involved in the pathogenesis of polyarthritis may exhibit a restricted use of TCR Vβ chains (19). The second study has utilized a PCR technique to examine TCR Vβ gene usage in cells obtained from the joints as well as LNs of B10.Q mice injected with chicken collagen type II (C.CII) (20). The authors observed that, whereas the joint T cells expressed Vβ2, Vβ6, Vβ8.2, Vβ9, Vβ10, and Vβ15 transcripts, the LN cells showed Vβ6, Vβ8.2, and Vβ9 transcripts. Although there are at least six different Vβ gene subfamilies expressed in the afflicted joints, and neither the specificity nor the clinical significance of the T cells expressing the above Vβ gene elements is known, the authors claimed that there is restrictive usage of TCR Vβ segments in CIA.

The present study aims at determining in more detail the TCR-α/β repertoire of the T cell response to B.CII that may play an important role in the induction of arthritis in DBA/1Lac.J (H-2^q) mice immunized with B.CII. 13 clonally distinct T cell hybridomas specific for B.CII have been established. These T cell hybridomas exhibit a highly restricted TCR-α/β repertoire. Accordingly, we were able to ameliorate CIA disease in mice by the use of TCR Vβ8.2-specific Ab.

Materials and Methods

Mice. DBA/1Lac.J, BALB/cJ, C57BL/6J, and SWR/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME).

Establishment of B.CII-specific T Cell Hybridomas. Male DBA/1Lac.J mice were immunized intradermally with 100 μg native B.CII (Elastin Products Company Inc., Owensville, MO) in CFA. Inguinal and popliteal LNs from three to four mice were removed 10 d later, and a single cell suspension was made in a serum-free medium (HL-1; Ventrex Laboratories, Portland, ME). Cells were plated at 4×10^6 cells per ml in a 24-well plate in the presence of 10 μg/ml B.CII for 3 d. T cells were expanded with IL-2 (20 U/ml) (Genzyme Corp., Boston, MA) for 2 d. Activated T cells were then fused with the TCR-α/β negative variant of the AKR thymoma BW5147 (a kind gift of Dr. Born, University of Colorado Health Sciences Center, Denver, CO), as described previously (21). A large number of hybrids were generated and screened for their reactivity against B.CII. These T cell hybridomas were then subcloned by limiting dilution (0.3 cell/well) and tested for their specificity against B.CII, C.CII (Genzyme Corp.), and B.CI (Sigma Chemical Co., St. Louis, MO) proteins. Stimulations of T cell hybrids were performed in triplicate 200-μl cultures containing 10^5 hybridoma cells, 3×10^5 DBA/1Lac.J splenocytes, and 2 μg collagen. After 24 h, culture supernatants were assayed for their ability to support the growth of 8×10^3 IL-2-dependent CTLL2 cells (American Type Culture Collection, Rockville, MD). Cell growth was assayed by the colorimetric MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (Sigma Chemical Co.) assay (22), and plotted as relative OD at 570/650 nm. IL-2 production by T cell hybridomas was determined from a standard curve generated with known quantities of recombinant mouse IL-2.

Flow Cytometric Analysis. The hybridoma F23.2 (mouse IgG1) specific for the mouse TCR Vβ8.2 (23) was a gift of Dr. Bevan (University of Washington, Seattle, WA). Biotinylated F23.2 mAb was a gift of Dr. Goverman (Caltech, Pasadena, CA). The hybridoma 44.22.1 (rat IgG2a) specific for the mouse TCR Vβ6 (24) was a gift of Dr. Hengartner (Institute for Pathology, Zurich, Switzerland). The purified F23.2 and 44.22.1 mAbs were purified from ascites fluid on protein G membranes (Amicon Corp., Beverly, MA). The hybridomas KT50 (rat IgG2a) and KT65 (rat IgG2a) specific for the mouse TCR Vβ8 (25) were a gift of Dr. Tomonari (Medical Research Council Clinical Research Centre, Harrow, Middlesex, UK). The hybridoma RR8-1 (rat IgG) specific for the mouse TCR Vα11 (26) was provided by Dr. Kanagawa. 10^6 T cells were stained with an anti-TCR V mAb supernatant followed by a FITC-conjugated goat anti-mouse IgG or goat anti-rat IgG Abs (Organon Teknika Corp., Durham, NC) and then subjected to flow cytometric analysis using a flow cytometer (50H Cytofluorograph; Ortho Diagnostic Systems Inc., Westwood, MA) as described previously (16). To determine the efficiency of in vivo depletion of T cells expressing TCR Vβ8.2, mice were given intraperitoneal injections of the F23.2 mAb (0.5 mg purified Ab per mouse). Draining LNs were removed 3 d after injection, a single cell suspension was made, and RBCs and dead cells removed using a lympholyte-M gradient (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada). To reduce background staining due to nonspecific binding, 10^6 lymphoid cells were resuspended in PBS containing 0.02% NaN_3, 2% normal mouse serum, and anti-CD32 mAb (Pharmingen, San Diego, CA). Lymphoid cells were stained with a biotinylated F23.2 mAb followed by a FITC-conjugated anti-mouse CD3 Ab (Boehringer Mannheim Biochemicals, Indianapolis, IN), and R-PE-conjugated avidin (Caltag Laboratories, San Francisco, CA). Lymphoid cells were then subjected to flow cytometric analysis using a flow cytometer (Epics Elite; Coulter Electronics Inc., Miami, FL).

Southern Blot Analysis. Southern blot analysis was carried out as described previously (27). The probe used to identify the Vα22 gene subfamily was made from the PCR product obtained from the amplification of a cDNA clone containing the TCR α chain gene of the 57.7 T cell hybridoma using two primers derived from the Vα22.1 gene segment: the sequence of the 5′ primer was 5′ CCGAATTACTTCTGGTGGTAC 3′, whereas the sequence of the 3′ primer was 5′ TGCTGCTGCACAGAAGTAGAT 3′.

PCR Amplification and Sequence Analysis. Total cellular RNA were isolated from the B.C II-specific T cell hybridomas (28). First-strand cDNA synthesis of TCR α or β chain gene was performed using Cα primer (5′ AGAGGGTGCTGTCCTGAGAC 3′) or Cβ primer (5′ GCCAAGCACACGAGGGTAGCC 3′). The reverse transcription reaction was performed in a DNA thermal cycler (Perkin-Elmer Cetus Corp., Norwalk, CT). The synthesized first-strand cDNA containing TCR-α or -β was then used as a template for the PCR amplification reaction. To amplify unknown Vα and Vβ genes expressed by the T cell hybridomas, three 5′ consensus Vα primers (5′ VαA: 5′ CTTCTGGTGGTACAGACA 3′, 5′ VαB: 5′ TCCTTTTCTGGTATGTGCA 3′, and 5′ VαC: 5′ AGCTGCAGTGGTTCCAACA 3′), and a single 5′ consensus Vβ primer (5′ ATGTACTGGTATCAGCAG 3′) were designed from all known Vα and Vβ gene segment sequences and used with the above Cα and Cβ primers. After amplification, T4 polymerase was then added and incubated at 37°C for 10 min. The PCR-amplified TCR-α or -β gene product was electrophoresed through 1.2% low-melting agarose gel and the DNA corresponding to the predicted size was isolated. The purified fragment was phosphorylated with a polynucleotide kinase and cloned into a blunt-ended dephosphorylated M13mp8 vector. Recombinant M13 were isolated and DNA sequence analysis was performed using the chain termination method (Sequenase version 2.0 kit; U.S. Biochemical, Cleveland, OH).

Results

Characterization of B.CII-specific T Cell Hybridomas. B.CII-reactive T cell hybrids were generated from three separate fusions. 13 were subcloned by limiting dilution (0.3 cell/well) for this study and their TCR α and β chain genes were subjected to sequence analysis. These T cell hybridomas were tested for their specificity against the B.CII, C.CII, and B.CI proteins. As shown in Fig. 1, all hybrids responded to both B.CII and C.CII, but not to B.CI. The low response of hybridoma 92 is due to the frequent loss of chromosomes encoding its TCR α/β chains in culture. Attempts to isolate a 92 hybridoma subclone expressing a high level of TCRs by limiting dilution has failed. These results indicate that the TCRs of these T cell hybridomas recognize conserved epitopes that are shared by type II collagens from different species and not by type I collagens. Hence, the T cell hybrids are protein type specific but not species specific.

Sequences of the TCR α/β Chain Genes. To examine heterogeneity of the T cell response to B.CII in CIA autoimmune disease, the TCR-α/β genes of the 13 B.CII-specific T cell hybridomas were subjected to sequence analysis using a sensitive and rapid PCR technique. On the basis of conserved 5′ DNA sequences present in all known Vα and Vβ gene segments, we designed 5′ Vα and 5′ Vβ oligonucleotide primers and used them with 3′ constant region Cα and and 3′ constant region Cβ primers, respectively, to amplify cDNA from B.CII-specific T cell clones expressing unknown TCR V region genes (see Materials and Methods). These T cell hybrids have been derived from different mice and three independent fusion experiments, and therefore, the T cell hybridomas are a representative panel of B.CII-specific T cell clones.

T cell clones can be divided based on Vα gene segment usage into three groups (Fig. 2). The first group employs

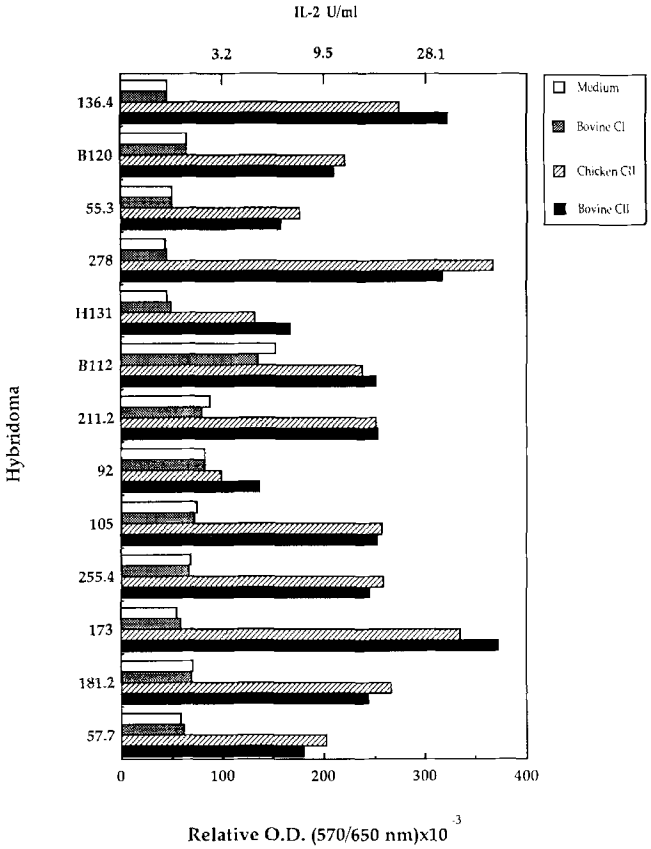

Figure 1. Response of the T cell hybridomas to B.CII, B.CI, and C.CII. Stimulations of T cell hybrids were performed in triplicate 200 μl cultures containing 10^5 hybridoma cells, 3×10^5 DBA/1Lac.J splenocytes, and 2 μg collagen. After 24 h, culture supernatants were assayed for their ability to support the growth of 8×10^3 IL-2-dependent CTLL2 cells in 96 microtiter plates. Cell growth was assessed after 24 h of culture by the colorimetric MTT assay and is plotted as relative OD at 570/650 nm. Units of IL-2 were deduced from the equation of a straight line (y = 56,103 + 212.51 log x, where y = absorbance U at 570/650 nm and x = IL-2 U) of a standard curve generated with known quantities of recombinant mouse IL-2.

two members of the Vα11 subfamily (~8–10 Vα members), Vα11.1^a, and Vα11.1^b. The difference between Vα11.1^a and Vα11.1^b regions is 11 nucleotides at the DNA level, and only five amino acids at the protein level. Because of the extensive homology between Vα11.1^a and Vα11.1^b gene segments, the Vα11.1^b gene segment was considered previously as an allele to the Vα11.1^a gene segment (26). Hence, the presence of both Vα11.1^a and Vα11.1^b gene segments in the DBA/1Lac.J genome, unequivocally indicates that they are not two allelic forms of the same gene segment, but rather two different members of the Vα11 subfamily. Consequently, a new nomenclature is proposed for these two gene segments: Vα11.1 and Vα11.4 to replace the old nomenclature, Vα11.1^a and Vα11.1^b, respectively. As noted in Fig. 2, two Jα gene segments (Jα42 and Jα37) were used in this group. The Jα42 segment is a newly identified functional Jα (29) and is preferentially selected by the rearranged Vα11.1, 11.4 gene segments (five of seven). In the second group, all three

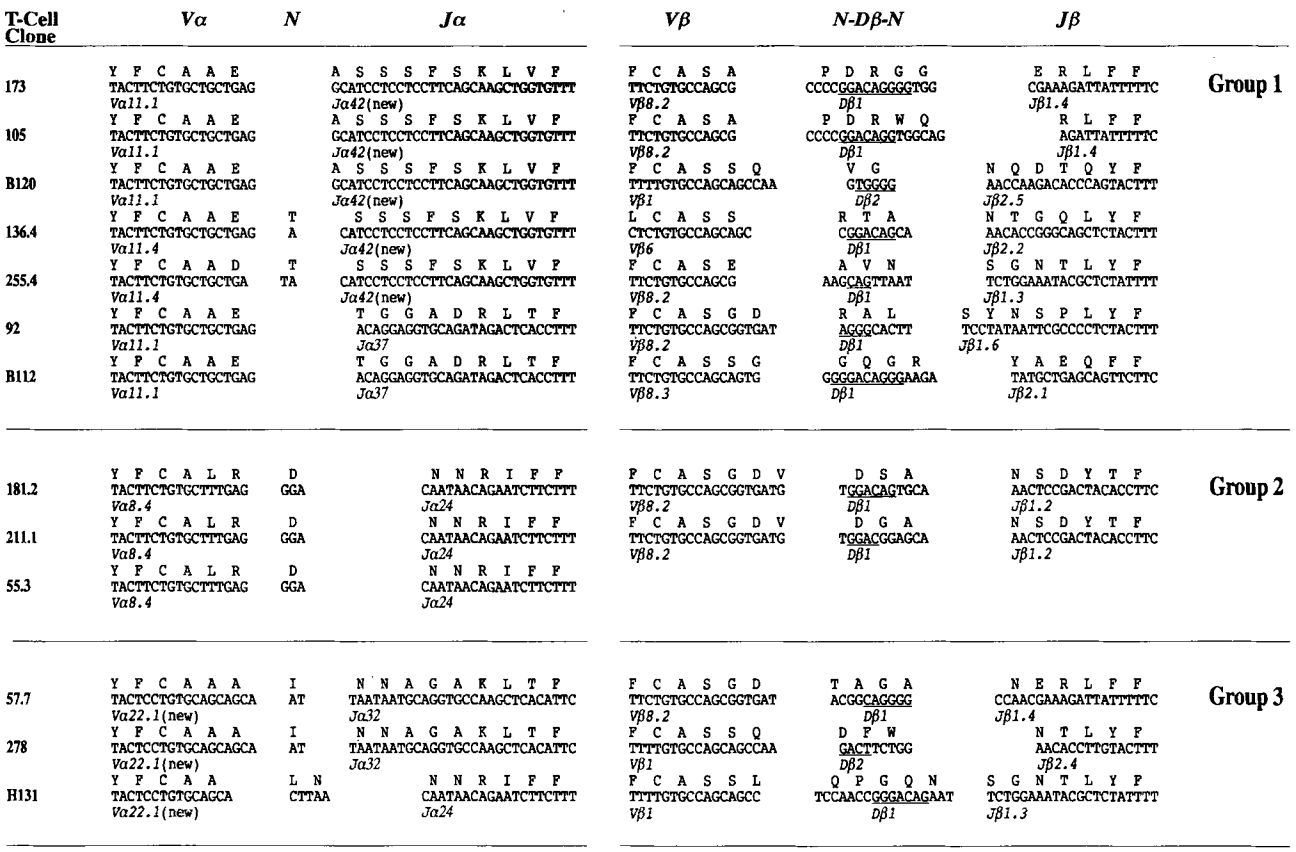

Figure 2. Nucleotide and amino acid sequences of Vα-Jα and Vβ-Dβ-Jβ junctional regions of the TCR α and β chain genes expressed by 13 T cell hybridomas specific for B.CII. On the basis of the TCR α chain usage, the T cell hybrid clones were divided into three groups. The 3′ boundaries of Vα 11.1, Vα 11.4, Vα 22.1, and Vβ1 gene segments were arbitrarily determined since germline sequence informations are not yet known. The remains of the germline Dβ1 (5′ GGGACAGGGGGC 3′), and Dβ2 (5′ GGGACTGGGGGGGC 3′) sequences are underlined. Nucleotides between Vα and Jα as well as Vβ, Dβ, and Jβ are proposed N region insertions. The predicted amino acid sequence is given above the nucleotide sequence using the single letter code.

T cell clones share the same Vα8.4 and Jα24 gene segments. The Vα8.4 gene segment is a member of the Vα8 subfamily which consists of ~8–10 members (30). Finally, the third group uses a new Vα gene segment, Vα22.1, which is distinct from the other 21 Vα subfamilies that have been previously described (Wang, K., J. L. Klotz, G. Kiser, G. Bristol, E. Lai, E. Gese, M. Kronenberg, and L. Hood, manuscript in preparation). A partial sequence of the Vα22.1 gene is given in Fig. 3 *A*. Genomic Southern blot analysis was performed to estimate the size of the newly identified Vα22 gene subfamily and to study the RFLP pattern among four inbred mouse strains of distinct Vα haplotypes: BALB/c ($V\alpha^a$), C57BL/6 ($V\alpha^b$), SWR ($V\alpha^c$), and DBA/1 ($V\alpha^d$) (26, 31). The result indicates that the Vα22 subfamily consists of approximately four to six members (Fig. 3 *B*). In addition, the Vα22 gene subfamily exhibits a high degree of polymorphism as indicated by the RFLP patterns generated using EcoRI and BamHI restriction enzymes (Fig. 3 *B*).

As shown in Fig. 2, the first group employs three Vβ gene subfamilies, Vβ1, Vβ6, and Vβ8. Two members of the Vβ8 subfamily were used, Vβ8.2 and Vβ8.3. However, the Vβ8.2 gene segment was found to be preferentially utilized (four of seven). Furthermore, seven distinct Jβ gene segments of the available 12 functional Jβ gene segments were used by the TCRs of the B.CII–reactive T cell hybridomas. Thus, unlike the biased Vβ8.2 gene segment usage in this group, the use of Jβ gene segments is more heterogeneous. In the second group, the T cell hybrid clones share the same Vβ8.2 and Jβ1.2. Finally, the third group uses Vβ1 and Vβ8.2 gene segments and three distinct Jβ gene segments.

Vα-Jα and Vβ-Dβ-Jβ Junctional Regions. A comparison of the nucleotide and predicted amino acid sequences of the TCR α/β chain junctional regions expressed by B.CII–specific T cell hybridomas (Fig. 2) reveals that there is a strong selection for highly conserved Vα-Jα junctional regions within each group. In contrast, Vβ-Dβ-Jβ junctional regions are generally diverse (Fig. 2). Another interesting observation is that several TCRs share an identical α chain in association with different β chains, e.g., clones 105, B120 (Vα11.1/Vβ8.2 and Vα11.1/Vβ1), clones 57.7, 278 (Vα22.1/Vβ8.2 and Vα22.1/Vβ1), and clones 92, B112 (Vα11.1/Vβ8.2 and Vα11.1/Vβ8.3). This indicates the strong in vivo selection by the antigen for either a particular α chain or a combination of both α and β chains.

Prevention of CIA in DBA/1Lac.J Male Mice Using mAbs Specific for TCR Vβ Chains. Since α and β chain sequences

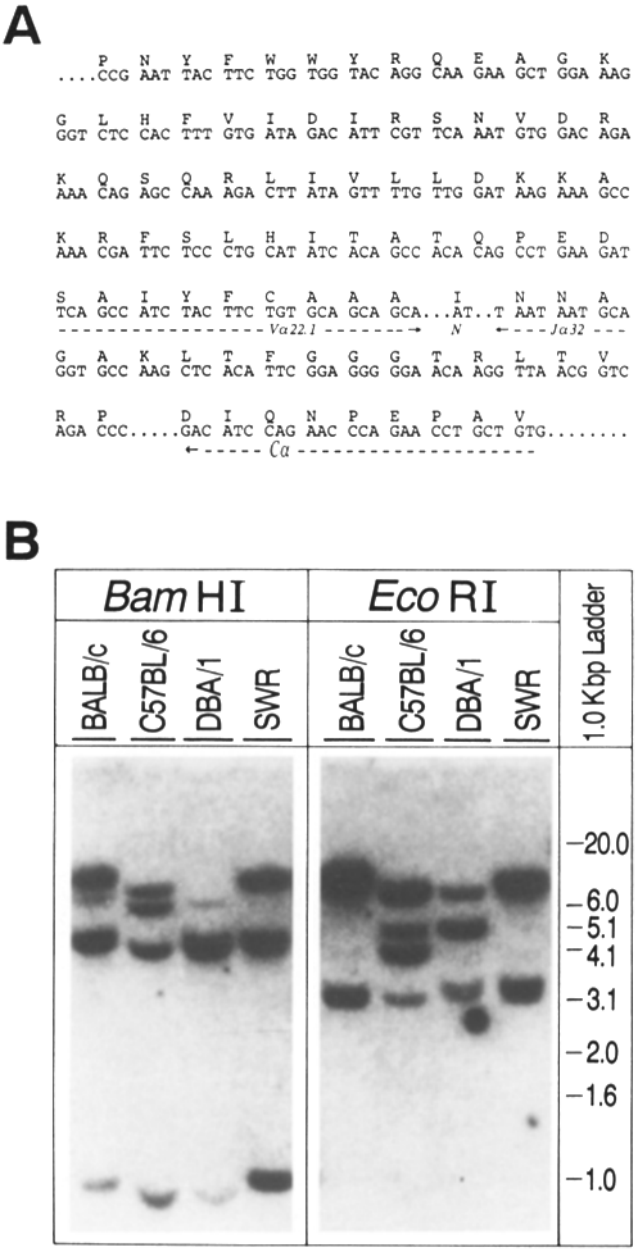

Figure 3. Partial nucleotide and predicted amino acid sequences of Vα22.1 region gene, and genomic Southern blot analysis of the Vα22 gene subfamily. (*A*) Partial nucleotide and predicted amino acid sequences of Vα22.1 cDNA clone derived from 57.7 T cell hybridoma specific for B.CII. Regions corresponding to Vα, Jα, and Cα are indicated. The 3' boundary of the Vα22.1 gene segment was arbitrarily determined since germline sequences of this new Vα subfamily are not yet known. Nucleotides between Vα and Jα gene segments are proposed N region insertions. The predicted amino acid sequence is given above the nucleotide sequence using the single letter code. The nucleotide sequence data will appear in the EMBL, Genbank, and DDBJ Nucleotide Sequence Databases (accession number X67949). (*B*) Genomic DNA were obtained from livers of BALB/c, C57BL/6, DBA/1Lac.J, and SWR/J mice. ~10 μg of EcoRI or BamHI-digested DNA was electrophoresed through a 0.7% agarose gel, transferred to a zeta-probe membrane, hybridized with a labeled Vα22.1 probe, and exposed to an x-ray film for 3 d at −80°C with an intensifying screen.

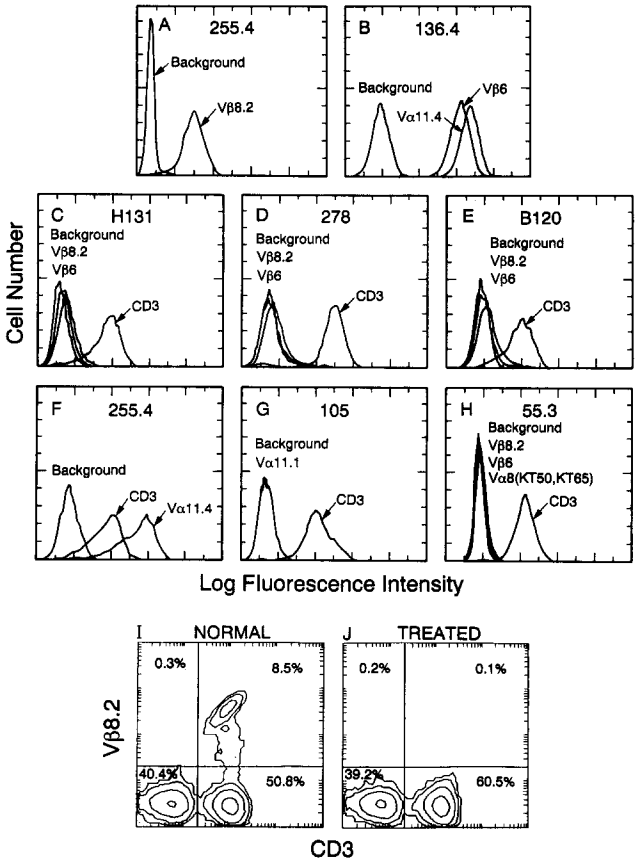

Figure 4. Cell surface expression of Vβ8.2, Vβ6, and Vα11.4 chains by a representative panel of T cell hybridomas specific for B.CII and the in vivo depletion of DBA/1Lac.J LN cells expressing TCR Vβ8.2 chain after intraperitoneal injection of F23.2 mAb. (*A–H*) T cell hybridomas 255.4, 136.4, H131, 278, B120, 105, and 55.3 were subjected to TCR surface expression analysis using fluorescence flow cytometry. The cells were stained with the mAb F23.2, 44.22.1, RR8.1, KT50, KT65, or anti-CD3 mAb followed by FITC-conjugated goat anti-mouse IgG or FITC-conjugated goat anti-rat IgG Abs. Background fluorescence represents staining of T cells with the FITC-conjugated Ab alone. Each histogram represents the analysis of 10,000 cells. (*I* and *J*) DBA/1Lac.J mice were injected with 0.5 mg i.p. F23.2 Ab. LN cells were removed after 3 d from treated mice (*J*) as well as normal mice (*I*) and stained with both biotinylated F23.2 and FITC-conjugated anti-CD3 mAbs in the presence of anti-CD32 mAb followed by R-PE-conjugated avidin. The two-color staining data are presented as dual-parameter contour plots in which increasing green fluorescence intensity (*CD3*) is plotted on the x axis versus increasing red fluorescence intensity (*Vβ8.2*) on the y axis. The percent values are the result of calculating the percentage of all cells within the region of interest.

of the TCRs of B.CII-specific T cell hybridomas have established that only three TCR Vβ gene subfamilies (Vβ1, Vβ6, and Vβ8) and three TCR Vα subfamilies (Vα8, Vα11, and Vα22) are employed, an examination of whether mAbs specific for TCR α chains and/or β chains can prevent CIA in mice was conducted. Although mAbs specific for TCR Vβ6, Vβ8, Vα8, and Vα11 are available, anti-TCR Vα8, as well as anti-TCR Vα11 Abs, are poorly characterized because of the complexity of both Vα8 and Vα11 gene subfamilies (~8–10 members each). Before pursuing our specific immune intervention experiment, the reactivity pattern of a panel of the

available mAbs against the B.CII-specific T cell hybrids was determined. The F23.2 mAb stained all T cell clones expressing TCR Vβ8.2. A representative of the T cell clones expressing TCR Vβ8.2 is shown in Fig. 4 *A*. In addition, 44.22.1 mAb recognized the TCR Vβ6 chain expressed by the clone 136.4 (Fig. 4 *B*). By contrast, neither F23.2 nor 44.22.1 recognized TCR β chains displayed by the clones H131, 278, and B120 (Fig. 4, *C–E*). This is consistent with the DNA sequence data (Fig. 2), indicating that the three clones express the TCR Vβ1 chain gene. The Ab RR8-1 stained the T cell clones expressing the TCR Vα11.4, but not the TCR Vα11.1 (Fig. 4, *F* and *G*). Finally, KT50 and KT65 mAbs specific for the TCR Vα8 chain failed to recognize the TCR Vα8.4 chain that is employed by three distinct T cell clones (Figs. 2 and 4 *H*). Therefore, neither anti-Vα11 nor anti-Vα8 mAbs can be used in our attempt to block CIA since both Abs failed to recognize the corresponding TCRs displayed by the majority of B.CII-reactive T cell hybrid clones. Therefore, the anti-Vβ8.2 and the anti-Vβ6 are the mAbs of choice for intervention experiments since neither anti-Vβ1 nor appropriate anti-Vα mAbs are yet available.

T cells reactive with the F23.2 mAb comprise 8–9% of T lymphocytes in the LNs of a normal DBA/1Lac.J mouse (Fig. 4 *I*). To examine the efficacy of in vivo depletion of T cells expressing TCR Vβ8.2, an intraperitoneal injection of 0.5 mg F23.2 Ab per mouse was found to be extremely efficient in eliminating virtually all TCR Vβ8.2-bearing T cells in LNs after 3 d (Fig. 4 *J*). The first question addressed was whether CIA can be blocked in DBA/1Lac.J mice with anti-Vβ8.2 Ab treatment. Mice received anti-Vβ8.2 Ab treatment twice: initially, 3 d before the intradermal primary immunization with B.CII in CFA, and the second time before the intraperitoneal boost on day 21. As shown in Table 1, only 6 of 20 mice treated with anti-Vβ8.2 Ab developed arthritis (30%), whereas 18 of 20 mice in the untreated group (90%), as well as 9 of 10 mice in the mouse IgG1 (MOPC21) treated group (90%) developed chronic polyarthritis.

Second, we were interested in examining whether mice treated with both anti-Vβ8.2 and anti-Vβ6 Abs would exhibit a reduced incidence of arthritis as compared with those pretreated with anti-Vβ8.2 Ab alone. Table 1 indicates that there is no significant difference between mice that received anti-Vβ8.2 Ab alone and those given anti-Vβ6 together with anti-Vβ8.2 Abs. Evidently, anti-Vβ8.2 Ab treatment alone is sufficient to reduce the incidence of arthritis by 60% in DBA/1Lac.J mice immunized with B.CII.

Discussion

CIA in mice is MHC class II restricted and a CD4 T cell–dependent autoimmune disease (10, 12). CIA resembles human rheumatoid arthritis in many clinical, histological, and immunological respects. These remarkable similarities make CIA an ideal model system for study. The experiments described in this report endeavored to determine the TCR-α/β repertoire in the CIA autoimmune disease model. 13 clonally distinct T cell hybridomas specific for B.CII have been established, and the α and β chain gene usage of their TCRs have been determined.

The T cell hybridomas recognized conserved epitopes, present only in collagen type II molecules from different species, and exhibited a limited TCR-α/β repertoire. Although T cell clones displayed three TCR β chains (Vβ8, Vβ1, and Vβ6), the Vβ8.2 gene segment is preferentially employed by the B.CII-specific T cell hybrid clones (58%) (Fig. 2). Unlike the Vβ gene segment usage, the Vβ-Dβ-Jβ junctional regions utilized are more heterogeneous (Fig. 2). Therefore, the repeated usage of two Vβ gene segments (Vβ1, 25% and Vβ8.2, 58.3%), since both Vβ6 and Vβ8.3 were used only once, strongly indicates that the TCR-β repertoire of

Table 1. *Inhibition of CIA in DBA/1Lac.J Male Mice Using anti-TCR β Chain Abs*

Antibody treatment	CIA incidence (%)	Average severity in afflicted mice mean ± SD	Day of onset
None*	18/20 (90)‡	3.94 ± 0.89§	49‖
Mouse IgG1 (MOPC21)	9/10 (90)	3.89 ± 0.33	49
Anti-Vβ8.2	6/20 (30)¶	3.00 ± 0.63	56
Anti-Vβ8.2 plus anti-Vβ6	7/20 (35)**	3.42 ± 0.97	56

* Four groups of DBA/1Lac.J male mice were treated intraperitoneally with the corresponding Abs (0.5 mg/mouse), and immunized 3 d later in the base of the tail with 100 μg of B.CII in CFA. On day 18, mice received another Ab treatment. Mice were challenged intraperitoneally with 100 μg B.CII in 100 mM acetic acid on day 21 and observed every other day for signs of arthritis.

‡ Number and percentage of mice that developed chronic polyarthritis.

§ Average clinical severity of mice with arthritis. Clinical severity in each affected paw is graded as: 0, normal; 1, redness and swelling; 2, deformity; 3, ankylosis. The scores were added to obtain the maximum arthritic score per mouse.

‖ The first day of detecting clinical symptoms of arthritis in mice. Mice were observed for the development of arthritis for up to 90 d.

¶ $p < 0.01$ when compared with the nontreated group using χ^2 test with Yates correction.

** $p < 0.01$ when compared with the nontreated group using χ^2 text with Yates correction.

the panel of the T cell hybridomas studied here is highly restricted. One plausible explanation for these highly restricted Vβ gene segments with diverse Vβ-Dβ-Jβ junctional regions is that the amino acid residues of Vβ1 and Vβ8.2 regions and not the Jβ, Dβ, or N region residues are primarily responsible for the interaction with collagen peptide–MHC complexes. In addition, of the three Vα gene subfamilies used by the TCRs of B.CII–reactive T cell hybridomas (Vα11, Vα8, and Vα22), the Vα11 gene subfamily is preferentially utilized (54%). Based on the Vα gene segment usage, the TCR α chains of the T cell clones are divided into three groups. Within each group, Vα-Jα junctional regions are highly conserved. Considering the powerful diversification mechanisms available for the TCR α/β chain genes, it is therefore obvious from the repeated usage of a few Vα gene segments (Vα11.1, 11.4, Vα8.4, and Vα22.1) of the estimated 100 distinct Vα gene segments available for the TCRs, that the B.CII as an antigen selects a restricted number of Vα gene segments. Another important point is that the selection of a particular Vα gene segment from a Vα subfamily that shares an extensive homology with the other multimembers of the same subfamily provides further evidence for the restricted use of Vα gene segments in the TCRs of the panel of B.CII–specific T cell hybrid clones. Similarly, the repeated employment of a few Jα gene segments (Jα42, Jα37, Jα24, and Jα32) of the available 40–50 functional Jα gene segments, strongly indicates that the Jα gene segment usage is also very restricted. Therefore, these results provide a compelling evidence for an exceedingly limited usage of Vβ, Vα, and Jα gene segments in the TCRs of B.CII–specific T cell hybridomas.

The resistance of the SWR mouse strain of the susceptible MHC haplotype (H-2^q) to CIA has been assigned either to its genomic deletion of 50% of the TCR Vβ gene segments, indicating the lack of expressing crucial Vβ genes (32), or to the deficiency of the C5 complement component (33). Our data demonstrating the effectiveness of anti-TCR Vβ8.2 Ab therapy in preventing CIA in DBA/1Lac.J mice, strongly suggest that the resistance of SWR mouse to arthritis may be due at least in part to the genomic deletion of the Vβ8.2 gene segment.

Akin to the EAE autoimmune disease model, CIA utilizes a highly restricted TCR-α/β repertoire and affords an opportunity for testing a specific immune manipulation. In establishing successful treatment of EAE, several specific immune intervention tactics were applied to eliminate or inactivate autoaggressive T cell clones. Among these approaches are: (*a*) the vaccination of Lewis rats against EAE by the use of synthetic peptides derived from either the hypervariable regions II or III of the TCR Vβ8.2 chain (34, 35); (*b*) the utilization of soluble class II MHC–MBP peptide complexes; prevention of EAE in SJL mice was accomplished by the intravenous injections of soluble complexes consisting of encephalitogenic peptide 91–103 of MBP and MHC class II ($I\text{-}A^s$) protein (36); and (*c*) the prevention of EAE in B10.PL mice by the use of a combination of anti-Vβ8.2 and anti-Vβ13 mAbs to eliminate the pathogenic T cells (16). Employing these therapeutic means towards CIA may prove to be successful. However, the first and second approaches are limited in their use because of required a priori knowledge. In the case of immunization with peptides derived from the TCR Vβ8.2, it is assumed that the host will elicit an immune response against that peptide, however this is not always the case since certain hosts may not be responsive to given peptides. As for using soluble MHC–peptide complexes, the identification of all different pathogenic T cell epitopes is a prerequisite. Since the anti-TCR Ab approach is not limited in the above respects, we believe that anti-TCR Ab therapy is an effective, therapeutic approach in preventing CIA as we have recently demonstrated in the case of EAE (16).

On the basis of the limited heterogeneity of the TCR α/β chains employed by the panel of T cell hybrid clones described here, examination of whether anti-TCR Ab therapy results in preventing the development of chronic polyarthritis, as in the case of the EAE autoimmune disease model, was possible. From our findings, it is evident that the anti-Vβ8.2 Ab treatment is significant in reducing the incidence of arthritis by 60% in DBA/1Lac.J mice immunized with B.CII, whereas the anti-Vβ6 Ab treatment does not result in any significant reduction of the disease incidence. One possible explanation for the failure of anti-Vβ6 treatment in reducing arthritis in mice, is that B.CII–specific Vβ6-expressing T cells may not play a clinically significant role in the induction of arthritis in mice. Another possibility is that the anti-Vβ6 mAb which is a rat IgG2a mAb, may not be effective in eliminating all Vβ6-bearing T cells as compared with the effective anti-Vβ8.2 mAb (mouse IgG1). Furthermore, it is not clear why some mice developed arthritis in spite of anti-Vβ8.2 treatment. One prospect is that these mice expand some other autoaggressive Vβ TCR–bearing cells because of the presence of a microenvironmental factor such as a bacterium or a virus. According to our sequence data, potential candidates are those expressing TCR Vβ1 chains. Unfortunately, anti-Vβ1 Ab is not available, and hence, it is not possible to examine the above prospect. Nonetheless, attempts to raise this Ab and to characterize the TCRs of the pathogenic T cell subset in the anti-Vβ8.2 treated mice are currently in progress.

In an attempt to address the issue that has been raised by Banerjee et al. (6) that certain TCR Vβ genes such as those which are deleted in the SWR mouse strain (37) play an important role in the induction of arthritis, Goldschmidt et al. (39) studied the effect of anti-TCR Ab treatment on CIA. They reported that the in vivo administration of anti-Vβ8.1, 8.2 or anti-Vβ6 Ab did not result in any significant alteration of CIA in DBA/1 mice immunized with rat collagen type II. In addition, their analysis of T cells obtained from Ab treated mice on day 21 revealed that 50% of Vβ8.1, 8.2–expressing T cells reemerged (38). Consequently, the efficiency of the Ab treatment in eliminating Vβ8.1,8.2–bearing T cells may account for the discrepancy between Goldschmidt group's and our findings. It is important to recall that in our study, mice received anti-Vβ8.2 Ab therapy twice; initially, 3 d before the primary immunization and the second time, on day 18. The rationale for this regimen is to ensure a very efficient depletion of Vβ8.2-expressing T cells over a long period of time. Therefore, the successful inhibition of CIA in DBA/1 mice may hinge upon the efficacy of the Ab treatment in

eliminating the autoaggressive Vβ8.2-bearing T cells. A similar observation has been documented by Chiocchia et al. (19).

In conclusion, analysis of the TCR α/β chains of our panel of B.CII-specific T cell hybridomas has demonstrated that the TCR-α/β repertoire is limited towards the utilization of a few Vα and Vβ gene segments. Furthermore, the anti-TCR therapy was found to be a very effective approach to significantly reduce the incidence of arthritis in DBA/1Lac.J mice. Nevertheless, a complete understanding of the molecular basis of the immune recognition in CIA depends fully upon the identification and characterization of different pathogenic collagen II peptides involved in the activation of the autoaggressive T cells. Thus, our panel of well-characterized T cell hybridomas is an extremely powerful tool to map and define the arthritogenic peptides involved in the disease induction. Currently, experiments designed to elute and sequence peptides bound in the MHC cleft using tandem mass spectrometry are in progress (39). Moreover, full characterization of the CIA autoimmune disease model may provide a basis towards future therapeutic strategies for preventive medicine in humans.

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