Expression of a Transgenic T Cell Receptor β Chain Enhances Collagen-induced Arthritis

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Summary

SWR/J transgenic (tg) mice were generated expressing the TCR β chain derived from an anti-collagen type II (CII) arthritogenic T cell clone. The SWR/J strain was selected because it is resistant to collagen-induced arthritis (CIA) and lacks the Vβ gene segment used by the T cell clone. Expression of the tg β chain on all thymocytes and peripheral lymph node T cells led to a more efficient anti-CII immune response, but did not confer CIA susceptibility to SWR/J mice. Nevertheless, this tg β chain enhanced predisposition to CIA as (DBA/1 × SWR) F_1 β tg mice were more susceptible than normal F_1 littermates. Our results demonstrate that the expression of the tg β chain contributes to CIA susceptibility, but by itself it is not sufficient to overcome CIA resistance in the SWR/J strain.

Collagen-induced arthritis (CIA)[1] develops in certain strains of mice, rats, and primates after immunization with native type II collagen (CII) in CFA (1–3). The disease can be induced by homologous as well as heterologous CII. Cyanogen bromide digestion of chicken CII gives rise to a major immunogenic and arthritogenic fraction which is represented by the CB11 peptide (4). Both antibody and T cell responses induced by heterologous CII immunization are predominantly directed to epitopes that are present in the CB11 peptide (5, 6).

In mice, susceptibility to the disease is associated with the expression of particular MHC class II alleles such as A^q and A^r (7, 8). Several polymorphic genes at other loci also influence disease susceptibility. The most extensively studied ones are genes encoding complement component C5, TCR β chain, and Mls antigens (9–12). Several investigators have attempted to clarify the putative role of C5 in susceptibility to CIA. The results are difficult to interpret and are in part contradictory (9–11).

The crucial role of T cells in CIA has been clearly documented in many studies (13). The disease can be transferred by T cells, either using cells freshly isolated from CII-immunized animals or using CII-specific Th cell lines and clones (14–16). Furthermore, CIA development can be prevented by injections of mAb directed against the CD4 and TCR molecules (17–19). Because of the linkage to particular class II alleles, one would expect that the "pathogenic T cells" may express a rather restricted TCR repertoire. Indeed, several findings suggest that the recognition of the disease-inducing CII epitope(s) requires particular TCR β chains. Mice with the susceptible H-2 background, but with a genomic deletion of certain TCR Vβ gene segments, are resistant to the induction of CIA. These mouse strains have a deletion encompassing the Vβ9, Vβ11, Vβ12, Vβ13, Vβ5, and Vβ8 gene families, and have been classified as $V\beta^a$ mutants (20, 21). Among these, SWR/J and AU/SSJ strains, both of the $H\text{-}2^q$ haplotype, are CIA resistant (22, 23). Also, RIII S/J mice, which have an even larger deletion including Vβ6, Vβ15, and Vβ17 genes ($V\beta^c$ mutants), are CIA resistant despite being $H\text{-}2^r$ (24). The correlation between resistance to the disease and deletion of certain Vβ genes suggests that these genes play a role in the pathogenesis of CIA. Although this hypothesis is supported by gene complementation and segregation experiments, the Vβ involvement in the control of CIA remains controversial (9–11, 22).

Recent studies on CIA in Mls-1 congenic mouse strains suggest that expression of a particular endogenous superantigen, $Mls\text{-}1^a$, results in decreased CIA incidence (12). Endogenous superantigens are characterized by their ability to be recognized by subsets of T lymphocytes bearing particular Vβ chains. Expression of the superantigen in the thymus of mice with a permissive H-2, results in clonal deletion of the T cells reactive to this superantigen. The consequence of the elimination of these autoreactive T cells is tolerance

[1] *Abbreviations used in this paper:* BCG, Bacillus Calmette-Guérin; CIA, collagen-induced arthritis; CII, collagen type II; tg, transgenic.

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to self superantigens (25, 26). With respect to CIA, the expression of Mls antigens may cause important deletions in the Vβ peripheral repertoire affecting T cells that are possibly important in the pathogenesis of the disease.

The TCR Vβ involvement in the pathogenesis of arthritic disease was also proposed upon analysis of TCR genes expressed in T lymphocytes present in the synovia of rheumatoid arthritis patients. In some (27–29), but not all of these studies (30–32), an expansion of oligoclonal T cells, or a restricted use of certain Vβ chains (33, 34) was observed.

In the present study we have analyzed the role of TCR Vβ genes in CIA using a different approach, namely by generating SWR/J mice that are transgenic (tg) for a TCR β gene derived from an anti-CII arthritogenic T cell clone. The particular T cell clone that we have used transfers the disease and expresses a Vβ region normally absent in the genome of the CIA-resistant strain, SWR/J (22). The tg SWR mice express the tg β chain on all T lymphocytes, and after CII immunization, show increased T cell–dependent anti-CII antibody responses, but do not develop CIA. However, introduction of the transgene into susceptible (DBA/1 × SWR/J)F_1 mice clearly increases CIA predisposition.

Materials and Methods

Mice. DBA/1J, SWR/J, and MORO/Ibm mice were obtained from Biological Research Laboratories, (Füllinsdorf, Switzerland). Transgenic mice were also bred and maintained in the mouse colony at Hoffmann-La Roche (Basel, Switzerland). F_1 crosses were made between SWR/J male mice heterozygous for the β transgene and DBA/1J female mice. For immunofluorescence analyses, unimmunized mice were killed at 4–8 wk of age and CII immunized at day 42 after immunization.

T Cells and Culture Conditions. Cells were grown in IMDM (Gibco, Paisley, UK) supplemented with 10% heat-inactivated FCS (Flow, UK), 2 mM L-glutamine, 1 mM Na pyruvate (Gibco), 5 × 10^{-5} M 2-ME (Sigma Chemical Co., St. Louis, MO), 50 U/ml penicillin, 50 μg/ml streptomycin, 100 μg/ml kanamycin (Gibco), and incubated in a humidified incubator with 5% CO_2. The anti-CII T cell clone K-102 was derived from the anti-CII T cell line previously described by Kakimoto et al. (15). The clone was characterized with respect to antigen specificity and shown to passively transfer arthritis in DBA/1 recipients (K. Kakimoto, unpublished results). K-102 cells (2 × 10^5/ml) were stimulated with 40 μg/ml human CII in the presence of 10^7/ml DBA/1 irradiated (3,000 rad) spleen cells. Between stimulations, cells were maintained in culture medium supplemented with 10% supernatant of Con A-stimulated rat spleen cells and 2 mg/ml methyl α-D-mannopyranoside (Sigma Chemical Co.) (35). 10 d after CII stimulation, blasts were purified over a ficoll gradient (Cederlane, Hornby, Canada) and fused with a subclone of BW-1100.129.237 $\alpha^-\beta^-$ lymphoma cells (36). T cell hybridomas were obtained after HAT selection, subcloned, and analyzed for antigen specificity, and CD3 and CD4 expression.

Collagens and Preparations of cyanogen bromide (CNBr) Digests of CII. Human and mouse type II collagens were purified from costal and sternal cartilages according to the method described by Miller et al. (37). The purity of CII was assessed by SDS-PAGE. Bovine CII was a kind gift from M. J. Mullqeen and D. Bradshaw (Roche Products, Welwyn Garden City, UK). Chicken CII was purchased from Genzyme Corp. (Boston, MA), bovine type I, human type III, and human type IV collagens were all purchased from Sigma Chemical Co. Bovine and chicken CII were digested with CNBr in 70% formic acid at 37°C. After desalting on a Sephadex G10 column in 0.1 M acetic acid, the digest was applied to a S-Sepharose column (Pharmacia, Uppsala, Sweden) equilibrated in 20 mM citrate, 20 mM NaCl pH 3.8 buffer, and eluted with a linear NaCl gradient (20–500 mM). The temperature of the column was maintained at 45°C. Fractions containing stimulatory activity in the functional assay (see below) were pooled, lyophilized, and subjected to gel filtration chromatography in PBS on a TSK 3000SW preparative column (LKB-Pharmacia, Gaithersburg, MD). The purity of the eluted peptides was checked by SDS-PAGE according to Laemmli (38), using a mini-gel system (Bio-Rad Laboratories, Richmond, CA). Partial amino acid sequences were determined on a protein sequencer (475 A; Applied Biosystems, Inc., Foster City, CA).

Antigen-specific IL-2 Release Assay. The T cell hybridoma (5 × 10^4 cells/well) was tested with a titration of collagens or CII CNBr peptides in the presence of DBA/1 or SWR/J spleen cells (5 × 10^5 cells/well) in flat-bottomed 96-well plates (Costar, Cambridge, MA). After 20–24 h of incubation, cell-free supernatants were collected and tested for IL-2 content using the CTLL-2 proliferation assay. CTLL-2 were plated at 5 × 10^3/well, and after 20–24 h of culture were pulsed for an additional 6 h with 1 μCi/well of [^{3}H]thymidine (Amersham International, Amersham, Bucks UK). Incorporated radioactivity was measured with a liquid scintillation β counter (Betaplate; LKB-Wallac, Turku, Finland) and is expressed as mean cpm of triplicate cultures.

Induction and Evaluation of CIA. Mice (8–10 wk) were immunized by intradermal injection in the back at the base of the tail with 200 μg of bovine or chicken CII in CFA (Difco Laboratories, Detroit, Michigan). An intraperitoneal injection of *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG, ~0.8–2.6 × 10^6 cells/mouse; Schweiz. Serum- & Impfinstitut, Bern, Switzerland) was also given to the mice at the same time. 21 d after the immunization, mice were boosted intraperitoneally with 200 μg of CII together with BCG. Arthritis was monitored daily from day 21 until day 35, weekly during the second month, and then every 2 wk thereafter. Mice were monitored for up to 3 mo, and in the case of SWR β tg mice, for up to 5 mo after immunization. Dates of onset of disease were recorded for individual mice. CIA was diagnosed if redness and swelling of fore- and hindpaws were observed. Severity of arthritis was evaluated for each paw and graded as following: grade 1, redness and swelling; grade 2, deformity; and grade 3, ankylosis. The scores for each paw were added to give an index ranging from 0 to 12 per mouse. None of the mice ever developed CIA after CFA/BCG injection. Statistical analysis was done using the χ^2 test (for comparing CIA incidence) and the Mann-Whitney test (for comparing day of onset and severity of CIA).

TCR β Gene Construct and Generation of SWR β tg Mice. The productively rearranged TCR β gene has been isolated by PCR amplification from genomic DNA of the anti-CII T cell clone. Primers used are complementary to sequences located in the 5′ flanking region of the Vβ12 leader sequence containing an additional SalI cloning site (5′ primer), and in the intron between Jβ1.2 and Jβ1.3 (3′ primer), allowing the amplification of a DNA region containing a BamHI site at the 3′ of Jβ1.2. PCR was performed for 35 cycles (94°C, 40 s; 65°C, 50 s; and 72°C, 60 s) followed by 5 min at 72°C, using Taq DNA polymerase (Perkin-Elmer Cetus, Emeryville, CA). The amplified fragment of ~900 bp was inserted into a plasmid vector containing TCR β enhancer and Vβ8.3 pro-

moter sequences (C. P. Gray, Hoffmann-La Roche, Basel, Switzerland). A 6.4-kb fragment spanning the region from the BamHI site located in the Jβ1 locus to the HindIII site at the end of the Cβ1 untranslated region, was further ligated into the construct at the corresponding BamHI site (see Fig. 2). This provides the entire Cβ1 region used by the K-102 T cell clone. After DNA sequencing to control possible PCR and ligation artifacts, the β construct was excised from the prokaryotic vector (pBluescript II KS⁻; Stratagene, La Jolla, CA) by digestion with BssHII, separated on agarose gel, and purified by phenol/chloroform extractions followed by ethanol precipitation. Transgenic mice were generated by microinjection of the purified DNA into SWR/J fertilized oocytes that were reimplanted into the oviducts of MORO/Ibm foster mothers as described (39). The integration of the transgene was analyzed both by PCR and Southern blot using DNA isolated from tail biopsies of 2–3-wk-old mice. The transcription of the full-length tg β RNA was demonstrated by Northern blot using total spleen RNA.

Antibodies and Flow Cytometry. The following mAb were used: anti-CD3 ϵ biotin-conjugated (500A2; PharMingen, San Diego, CA) or FITC-conjugated (145-2C11; Boehringer Mannheim, Mannheim, Germany); anti-CD4 PE-conjugated (GK1.5; Becton Dickinson & Co., Mountain View, CA); anti-CD8 FITC-conjugated (53-6.7; Becton Dickinson & Co.); anti-Thy 1.2 FITC-conjugated (30-H12; Becton Dickinson & Co.); anti-Vβ2 (B20.6.5, kindly provided by B. Malissen, Marseille-Luminy, France); anti-Vβ3 (KJ25a; J. Kappler, Howard Hughes Medical Institute, Denver, CO); anti-Vβ4 (KT4-3; K. Tomonari, Medical Research Council, Harrow, U.K.); anti-Vβ6 (44-22-1; H. Hengartner, University Hospital, Zürich, Switzerland); anti-Vβ8.1, 8.2, and 8.3 (U. Staerz, National Jewish Center, Denver, CO); anti-Vβ11 (KT11; K. Tomonari); and anti-Vβ17a (KJ23a, J. Kappler) were all used as cell culture supernatants. The MR11-1 hybridoma secreting anti-Vβ12 mAb was produced by O. Kanagawa (Washington University, St. Louis, MO), and obtained through the courtesy of H. R. MacDonald (Ludwig Institute for Cancer Research, Lausanne, Switzerland). The antibody was purified from the cell culture supernatant and biotinylated by standard procedures using *N*-hydroxysuccinimidobiotin (Sigma Chemical Co.). Second step reagents were: sheep anti-mouse Ig-FITC, and sheep anti-rat Ig-FITC (Silenus, Hawthorn, Australia); goat anti-mouse Ig-PE, and goat anti-rat Ig-PE (Southern Biotechnology Associates, Birmingham, AL); and streptavidin-FITC or streptavidin-conjugated tandem label of PE/Texas Red (Southern Biotechnology Associates). All incubations and washings were done at 4°C in PBS, 2% FCS, 0.02% NaN_3. 20,000 or 50,000 viable cells were analyzed by FACScan® (Becton Dickinson & Co.). Dead cells were excluded from the analysis using forward and side scatter parameters and also using propidium iodide when possible.

Serum Anti-CII Antibody Levels. ELISA was performed as follows: flat-bottomed Maxi Sorp 96-well Immuno Plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with 50 μl/well of either CII or purified protein derivative from *Mycobacterium tuberculosis* (PPD, Statens Seruminstitut, Copenhagen, Denmark), BSA (Sigma Chemical Co.), or goat anti-mouse Ig (Southern Biotechnology Associates), all at 10 μg/ml. Plates were saturated with 1% BSA in PBS, and after washing, sera were added at various dilutions. After five washes with PBS, 0.05% Tween 20, β galactosidase-labeled antibodies (anti-mouse Ig, IgM, IgG1, IgG2a, IgG2b, IgG3, Southern Biotechnology Associates) were added and incubated overnight at 4°C. Positive wells were revealed with *O*-nitrophenyl-β-D-galactopyranoside (Sigma Chemical Co.) and OD_{405nm} was determined using a microplate reader (Anthos Labtec, Basel, Switzerland).

Results

The T Cell Hybridoma BL17 Recognizes the CB11 Fragment of CII. A T cell hybridoma, BL17, was derived from the fusion of the arthritogenic anti-CII T cell clone K-102 and the BW-1100.129.237 $\alpha^-\beta^-$ T cell lymphoma (see Materials and Methods). This hybridoma expresses high levels of CD3 and CD4. Stimulation by immobilized anti-αβ TCR or anti-CD3 antibodies leads to IL-2 release and apoptosis (data not shown). Like the parental clone, the hybridoma produces IL-2 in response to bovine, chicken, human, and mouse CII presented by DBA/1 APC (data not shown).

To further characterize the antigen specificity, bovine and chicken CII were digested with CNBr, and the peptides were fractionated by ion exchange and gel filtration chromatography. The fractions were tested for their ability to induce IL-2 release in the presence of APC. Stimulatory activity could be attributed to a peptide migrating with an apparent molecular mass of about 36 kD on SDS-PAGE (data not shown). Partial amino acid sequences of the stimulatory peptides from both bovine and chicken CII showed that they were identical with the previously described CB11 peptide (40), a 279-amino acid-long peptide that is the main CII fragment recognized by both antibodies and T cells in mice with CIA.

SWR/J Spleen Cells Can Process and Present CII and its CB11 Fragment to the T Cell Hybridoma. SWR/J mice are not susceptible to CIA induction, perhaps because of the deletion of some Vβ gene segments. An alternative explanation could be that although they carry susceptibility-associated H-2 genes, their APC might be unable to present arthritogenic CII epitopes. We have excluded this possibility by showing that spleen cells from SWR/J mice are able to process and present either bovine, chicken and human CII (Fig. 1 *a*), or bovine and chicken CB11 (Fig. 1 *b*) to the BL17 hybridoma.

Thus, the resistance of SWR/J to CIA is not due to a defect in antigen presentation.

Generation of TCR β Transgenic Mice. We have attempted to sensitize the SWR mice to CIA by introducing a transgene encoding the BL17 TCR β chain. This TCR β chain was shown to be encoded by Vβ12-Dβ1.1-Jβ1.1 and Cβ1 (L. Mori, unpublished results). The productively rearranged

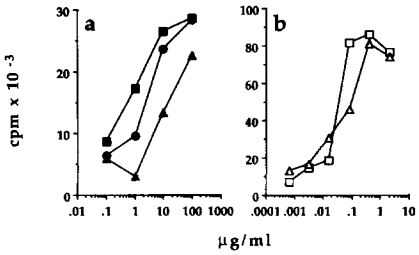

Figure 1. Antigen presentation by SWR/J spleen cells. BL17 cells were stimulated by CII or its CB11 fragment in the presence of SWR/J APC. The levels of IL-2 secreted were measured using the CTLL-2 assay. (*a*) Shows the stimulation with bovine CII (■), human CII (●), and chicken CII (▲); (*b*) with bovine CB11 (□) and chicken CB11 (△). Results are expressed as mean cpm of [³H]thymidine incorporation of triplicate cultures.

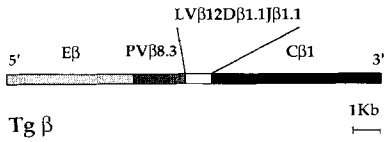

Figure 2. TCR β construct used for the generation of transgenic mice. The construct is composed of a PCR-amplified fragment that contains the leader sequence of Vβ12 (*L*), Vβ12, Dβ1.1, and Jβ1.1 (nucleotide sequence is available upon request). This fragment is inserted 3' to the β locus enhancer (*E*β) and promoter (*P*) of Vβ8.3, and 5' to the entire Cβ1 locus.

TCR β gene was isolated by PCR amplification of genomic DNA isolated from the anti-CII T cell clone K-102, and inserted into a plasmid vector containing the TCR β enhancer and Vβ8.3 promoter sequences. A fragment encompassing the entire Cβ1 locus was further inserted at the 3' end of the Vβ gene (Fig. 2). This TCR β construct, free from vector sequences, was used for the generation of tg mice.

Two tg mouse lines were obtained in the SWR/J strain. All experiments described in this study were carried out with one of these lines (SWR-βL tg) which integrated about 10 copies of the transgene into the genome and expressed very high levels of tg β chain mRNA in the spleen (data not shown).

Expression of the TCR β Transgene in the Absence of Endogenous β Genes. The expression of the TCR β transgene was tested using a mAb specific for the mouse Vβ12 polypeptide. Thymocytes and lymph node cells from tg mice and non-tg littermates were stained with mAb specific for CD4, CD8, and Vβ12, and evaluated by three-color FACS® analysis.

In the thymus, Vβ12$^+$ cells were undetectable in SWR mice, as expected, but were present in SWR-βL tg animals (Fig. 3, *a* and *d*). The number of Vβ12$^+$ and CD3$^+$ cells

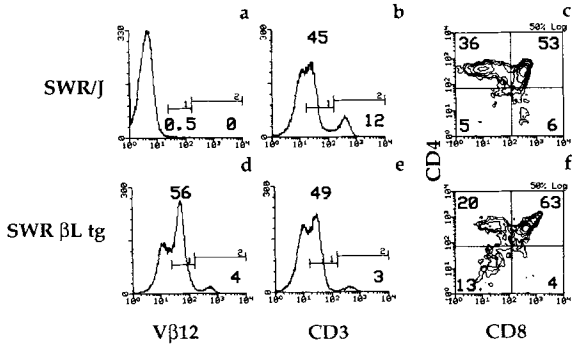

Figure 3. Thymocyte surface expression of the transgenic β chain in SWR-βL tg mice. Thymocytes from SWR-βL tg and non-tg, sex-matched, littermate mice were stained with anti-CD8 (FITC-conjugated), anti-CD4 (PE-conjugated) and anti-Vβ12 or anti-CD3 (biotin-conjugated), followed by labeling with streptavidin-tandem (see Materials and Methods). (*a* and *d*) Histograms of the Vβ12 staining; (*b* and *e*) Histograms of the CD3 staining; (*c* and *f*) Contour plots for CD4 and CD8 stainings of cells gated as CD3$^+$. Numbers represent the percentage of cells in each region. The difference in staining of the TCR^{low} population, using anti-Vβ12 or anti-CD3, has to be attributed to different affinities of the two mAb.

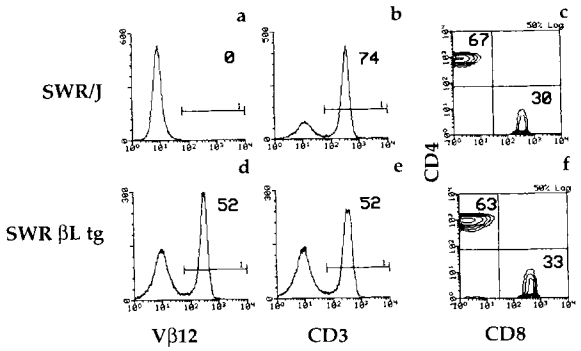

Figure 4. Lymph node T cell surface expression of the transgenic β chain in SWR-βL tg mice. Total lymph node cells from SWR-βL tg and non-tg, sex-matched, littermate mice were analyzed by three-color staining as in Fig. 3. (*a* and *d*) Histograms of the Vβ12 staining; (*b* and *e*) Histograms of the CD3 staining; (*c* and *f*) Contour plots for CD4 and CD8 stainings of cells gated as CD3$^+$. Numbers represent the percentage of cells in each region.

was similar, thus indicating that every T cell expresses the transgene. Although the total number of CD3$^+$ thymocytes was similar in normal and tg animals, there were differences in the percentages of mature cells. Tg mice had a lower number of CD3^{hi} thymocytes (3% vs 12% in normal animals) and this was compensated by a higher number of immature, CD3^{low} cells (49% vs 45%; Fig. 3, *b* and *e*) and CD3$^+$CD4$^+$CD8$^+$ cells (63% vs 53%; Fig. 3, *c* and *f*).

In lymph nodes of tg mice all CD3$^+$ cells were Vβ12$^+$ and were reduced in number (52% vs 74% of total lymph node cells; Fig. 4, *a*, *b*, *d*, and *e*), but the ratio of CD4 to CD8 cells was as in normal mice (Fig. 4, *c* and *f*).

We also used other Vβ-specific mAb (anti-Vβ 2, 3, 4, 6, and 17a) to show the lack of expression of endogenous β genes. Indeed, none of these reagents stained either thymocytes or lymph node cells in SWR-βL tg animals (data not shown).

These results show that the tg β chain is expressed on the surface of T cells and that it prevents the expression of the endogenous TCR β chains. Furthermore, expression of this chain also affects the number of mature cells, without altering the relative percentages of CD4$^+$ and CD8$^+$ subsets.

SWR-βL tg Mice Mount Strong Anti-CII Antibody Responses. The antibody response of mice to CII is strictly T cell dependent (41). SWR β tg and non-tg littermates, as well as DBA/1 mice were immunized with bovine CII in CFA. At different time points after immunization, mice were bled and the anti-CII antibodies were analyzed by ELISA. It is interesting that the SWR-βL tg mice produce more anti-CII antibodies (day 7 titer, 8; day 14 titer, 50; and day 21 titer, 400) than DBA/1 mice (day 14 titer, 15, and day 21 titer, 200) and SWR non-tg littermates (day 14 titer, 3, and day 21 titer, 25; Fig. 5). Furthermore, their response developed earlier as compared with the other groups. Already at day 7 after immunization, SWR-βL tg mice had detectable titers of anti-CII antibodies of the γ1, γ2b, and γ2a isotypes. The anti-CII antibody titer in SWR-βL tg mice was always

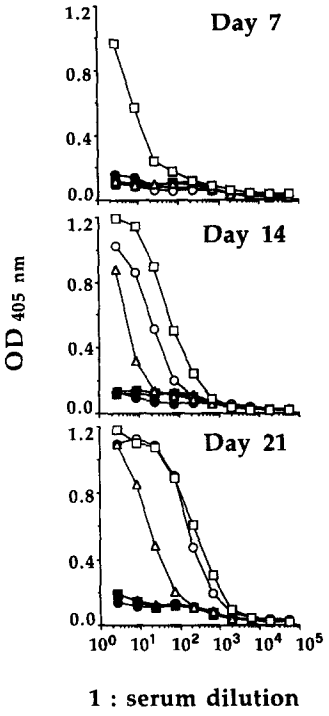

Figure 5. Increase in the total anti-CII antibody titer in the primary response of SWR-βL tg mice. Titration of the total anti-bovine CII Ig present in the serum of mice immunized with bovine CII in CFA (*open* symbols) or only injected with CFA (*closed* symbols) on different days after immunization: SWR-βL tg (□, ■); SWR/J (△, ▲); DBA/1 (○, ●). Each curve represents the values obtained from the pooled sera of three mice. Results are expressed in OD_{405nm}. Control ELISA with BSA-coated plates gave background values ≤0.172.

Table 1. *SWR-βL tg Mice are Resistant to CIA*

	Mice*	Arthritic/Total	Incidence
			%
Experiment 1	SWR-βL tg	0/12	0
	SWR/J	0/14	0
	DBA/1	7/11	64
Experiment 2	SWR-βL tg	0/13	0
	SWR/J	0/8	0
	DBA/1	6/7	86
Experiment 3	SWR-βL tg	0/6	0
	SWR/J	0/5	0
	DBA/1	4/5	80

* Male and female mice were used.

higher than in SWR non-tg littermates for all antibody classes and at all time points (data not shown).

Thus, in the SWR-βL tg mice there seems to be an early switch to all Ig isotypes and in particular to γ2a that predominates in the anti-CII antibody response during CIA (41). This finding, together with the fact that the antibody titer is higher in the tg mice, suggests that these mice may have an increased number of CII-specific Th cells.

SWR-βL tg Mice Are Resistant to CIA. SWR-βL tg mice were tested for the development of CIA, to determine whether the expression of a Vβ12 bearing TCR β chain on all T cells, and the increased production of anti-CII antibodies observed in these mice were sufficient to make them susceptible to the disease. A total of 31 SWR-βL tg mice and 27 SWR non-tg littermates were immunized with bovine CII in CFA in three separate experiments. 21 d after the immunization, mice were boosted with bovine CII and analyzed for the development of arthritis. As shown in Table 1, none of the SWR developed clinical signs of CIA regardless of the presence of the transgene, even 5 mo after the first injection. In contrast, 64–86% of the DBA/1 mice showed clinical signs of CIA, starting from day 21 to day 33 after CII immunization.

Expression of the tg β Chain in Susceptible Mice Leads to Increased Incidence and Severity of CIA. Susceptibility to CIA is a dominant trait since (DBA/1 × SWR) F_1 mice develop CIA (10, 11). Both tg and non-tg F_1 littermates, derived from crossing SWR mice heterozygous for the β tg with DBA/1, were immunized with CII and observed for the development of CIA. Arthritis developed in 38% of (DBA/1 × SWR) F_1 mice and in 89% of the F_1 βL tg mice in a total of three independent experiments (Table 2). The disease induced in F_1 βL tg mice was more severe (median arthritic score 8 vs 4) and developed 2 d earlier than in F_1 non-tg littermates. Vβ12 was expressed in the tg mice by all T cells present in lymph nodes draining the affected limbs, as well as in the corresponding lymph nodes of the tg mice without clinical CIA (Fig. 6). FACS® analysis using other Vβ-specific mAb (anti-Vβ 2, 3, 4, 6, 8, 11, and 17a), showed that none of the F_1 βL tg animals also expressed endogenous β genes (data not shown).

Table 2. *CIA in (DBA/1 × SWR-βL tg) F_1 Mice*

Mice	Arthritic/Total	Incidence*	Day of onset**	Severity***
		%		
F_1 βL tg littermates	25/28	89	26 (21–29)	8 (12–3)
F_1 non-tg littermates	8/21	38	28 (28–36)	4 (9–3)

* $p < 0.001$.
** Median values (range), $p < 0.01$.
*** Median values (range), $p < 0.05$.

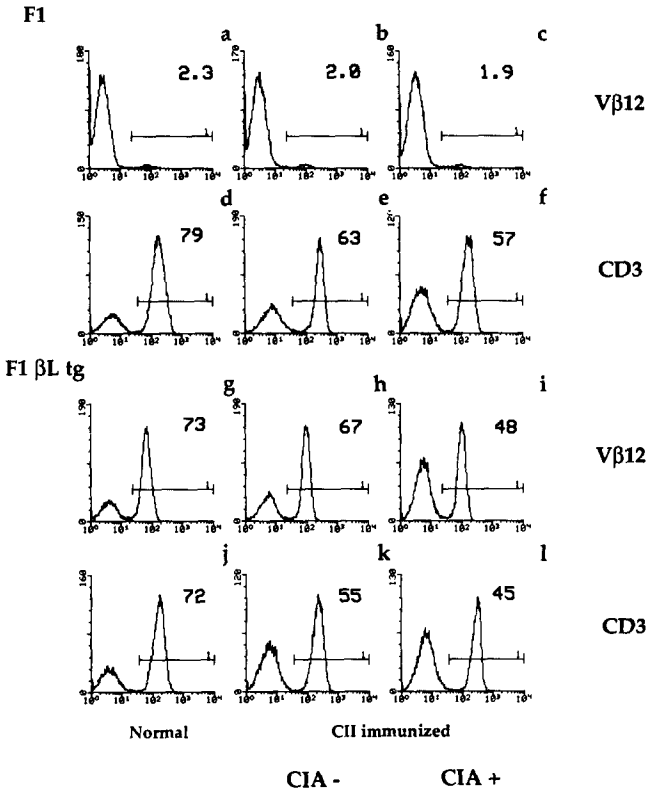

Figure 6. Lymph node T cell surface expression of the transgenic β chain in (DBA/1 × SWR-βL tg) F_1 mice. Total lymph node cells from F_1 βL tg and non-tg, sex-matched, littermate mice were stained with anti-Vβ12 biotin-conjugated followed by streptavidin-FITC (*a–c, g–i*) or with anti-CD3 FITC-conjugated (*d–f, j–l*) and analyzed by FACScan®. (*a, d, g, j*) Histograms of stained cells from mice before CII immunization; (*b, e, h, k*) Histograms of stained cells from CII-immunized mice without clinical CIA at day 42; (*c, f, i, l*) Histograms of stained cells from CII-immunized mice with clinical CIA at day 42. Numbers represent the percentage of cells in each region.

These results indicate that the expression on all T cells of a TCR β chain derived from an arthritogenic anti-CII T cell clone, increases susceptibility to CIA in prone (DBA/1 × SWR/J) F_1 mice, but does not confer susceptibility to resistant ones (SWR/J).

Discussion

Transgenic mice have been generated expressing the TCR β chain derived from the arthritogenic anti-CII T cell clone K-102. The clone responds to CII of several animal species, including mouse, and recognizes CB11, which is the main arthritogenic CII peptide. DBA/1 mice that are injected with K-102 cells acquire a mild form of arthritis (K. Kakimoto, unpublished results).

For the generation of tg mice, we have chosen the SWR/J strain because it is resistant to CIA and because it lacks the Vβ12 gene that is expressed by the T cell clone.

TCR β tg SWR mice express the tg β chain on the surface of immature double- and of mature single-positive thymocytes, without any preference for single $CD4^+$ cells. All thymocytes express only the tg β chain, which is in agreement with previous findings that showed that functionally rearranged TCR β transgene prevents the expression of endogenous β genes (42). In tg animals, the number of mature thymocytes that express high levels of TCR is reduced, as compared with thymocytes of normal SWR mice, but cells expressing low TCR levels are slightly increased. The reduction in the number of mature $\alpha\beta$ cells in the β tg mice is probably due to the limitation in TCR repertoire generation. This limitation could result from the lack of expression of more than one β chain, as well as from the failure of this β chain to pair with certain α chains (43).

Also, the T cells in peripheral lymph nodes of tg mice expressed only the tg β chain, as demonstrated by immunofluorescence using anti-CD3, anti-Vβ12, and a panel of anti-Vβ mAb. Despite the reduction in the TCR repertoire size and in the number of $\alpha\beta^+$ T cells, tg mice did not show any obvious signs of immunodeficiency. They are capable of developing protective immune responses against viruses and bacteria to a similar extent as their non-tg littermates (data not shown). When immunized with CII, the tg mice develop immunity to CII as demonstrated in vitro by the isolation of CII-specific T cells (data not shown) and in vivo by the production of anti-CII antibodies of all IgG classes. Furthermore, the antibody responses develops faster in tg than in non-tg mice, presumably because of the increased number of CII-specific Th cells.

Despite the very effective anti-CII immune response, SWR-βL tg mice do not develop CIA after CII immunization. Nevertheless, we could demonstrate a role for the Vβ12 tg chain in CIA as (DBA/1 × SWR) F_1 βL tg mice were clearly more susceptible than normal F_1 littermates. The development of CIA in F_1 βL tg mice that do not express endogenous β genes, rules out the possibility that T cells bearing β chains other than the tg β chain are required for the initiation and/or the progression of the disease.

Apparently, TCR β tg SWR mice lack other genes that are required for CIA susceptibility. What are these other genes? One possible candidate is the gene encoding the complement component C5. This gene is defective in SWR mice and its role in CIA remains controversial (9–11). Other candidate genes might be involved in antigen recognition and include the TCR α genes and the antigen itself. Differences in antigen processing and presentation that might exist in different mouse strains do not seem to be relevant, as we have demonstrated that SWR cells process and present the arthritogenic CII epitope to the T cell hybridoma. The possibility exists that SWR mice express a CII allele whose product is not recognized by the T cell clone that we have used as a source of the transgene. Alternatively, we cannot exclude the possibility that SWR mice lack a particular Vα gene segment that is required for the generation of T cells that cause arthritis, but not of Th cells involved in anti-CII antibody production. The generation of mice tg for both α and β genes derived from the arthritogenic clone will address this point.

In conclusion, our tg model has confirmed the importance

of the TCR β genes in the development of CIA, and has clearly shown that a tg β chain derived from an arthritogenic T cell clone can increase susceptibility to CIA. This tg model also indicates that other genes are involved in the pathogenesis of arthritis, thus adding CIA to the list of autoimmune diseases with multiple genetic control.

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