POSSIBLE ROLE OF V_β T CELL RECEPTOR GENES IN SUSCEPTIBILITY TO COLLAGEN-INDUCED ARTHRITIS IN MICE

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Collagen-induced arthritis (CIA)[1] is an autoimmune disease that develops after the immunization of susceptible strains of rodents (1, 2) with native type II collagen in Freund's adjuvant. Cellular and T cell–dependent humoral immune responses to type II collagen are both crucial to the pathogenesis of this disease (3).

The susceptibility to the development of arthritis is linked to the MHC complex in rodents (2, 4). It has been shown by us earlier that in mice only H-2^q and H-2^r haplotypes develop this disease (2, 5). In the H-2^q mice, this susceptibility has been further mapped to the I-A subregion by the use of recombinant strains (6). The role of non-MHC genes, however, was also suggested earlier (5) by the observation that SWR mice, though having an H-2^q haplotype, were totally resistant to CIA.

It was reported recently (7) that SWR mice have a genomic deletion of close to 50% of the genes coding for the variable region of the β chain of the T cell receptor (V_β TCR) in an already restricted V_β TCR gene repertoire in mice. There were no additional genes to replace those that were deleted. It was hypothesized by us then that the resistance of SWR mice to CIA could be related to the absence of T cells with TCR specificity directed towards the arthritogenic determinant(s) (8, 9) on the collagen molecule. To investigate this further, crosses were made between SWR and another CIA-resistant mouse B10 (H-2^b). The susceptibility to CIA was tested in H-2^q-bearing mice in the F_1 and F_2 hybrids, and in the B10 and SWR backcrosses. B10 mice (H-2^b) are CIA resistant due to a resistant H-2 gene (2) but have a wild-type TCR (7). In this paper, we outline the results of our preliminary findings which indicate that a normal set of V_β TCR genes are probably required in addition to the I-A^q genes for susceptibility to collagen-induced arthritis in H-2^q mice.

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[1] *Abbreviation used in this paper:* CIA, collagen-induced arthritis.

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Materials and Methods

Mice. SWR/J mice were purchased from The Jackson Laboratory, Bar Harbor, ME. B10 mice and the various crosses of B10 with SWR mice were made and bred in our mouse colony.

Induction of CIA. Native bovine type II collagen (BII) isolated from bovine articular cartilage (10) was used for immunization. Arthritis was induced by intradermal injection of 100 μg of native type II collagen in CFA (1:1) at the base of the tail followed by a boost of 100 μg of collagen at 3 wk. Mice were monitored at frequent intervals for the development of arthritis in peripheral joints up to 5 mo after primary immunization. The severity of arthritis in each affected paw was graded as grade 1, redness and swelling; grade 2, deformity; and grade 3, ankylosis in the affected joint (2). The maximum grades in the affected paws of each diseased mouse were added to give an arthritic score for the animal. The course of arthritis was ascertained by measurement of paw thickness by constant tension calipers (The Dyer Company, Lancaster, PA) and manipulation of affected joints for ankylosis.

Flow Cytometry. F23.1 hybridoma (12) (IgG2a,K) was a kind gift from Dr. J. Bluestone, National Institutes of Health, Bethesda, MD. The antibody binds to T cells expressing $V_\beta 8$ subfamily genes in the β chain of their TCR (13). $V_\beta 8$ TCR genes are some of the genes deleted in SWR mice (7). F23.1 was affinity purified and biotinylated by standard procedures. Peripheral blood (0.5 ml) was obtained from the tail veins of arthritic mice and the lymphocytes separated over a Lymphopaque (Nyegaard, Oslo, Norway) gradient and washed twice with 1% BSA in PBS, pH 7.2, containing 0.1% sodium azide. All dilutions and washings were made in this buffer. The lymphocytes were incubated with rat anti-mouse Thy 1.2 (Becton Dickinson Monoclonal Center, Inc., Mountain View, CA) for 30 min at 4°C, washed twice, and then further incubated with fluorescein-conjugated mouse anti-rat IgG (Accurate Chemical & Scientific Corp., Westbury, NY) for 30 min at 4°C. After two washes, these were further incubated with biotinylated F23.1 for 30 min at 37°C, washed twice, and then reincubated with streptavidin-phycoerythrin (Becton Dickinson Monoclonal Center, Inc.) at room temperature for 30 min. After further washing, the cells were analyzed on FACS IV (Becton Dickinson Immunocytometry Systems, Mountain View, CA) after gating on lymphocytes. In each run we included PBL from SWR mice as a negative control and B10 mice as a positive control.

Anti-Collagen II Antibodies. Serum total and IgG subclass-specific anti-type II collagen antibodies were estimated by standard ELISA methods (10).

Serotyping for H-2 Haplotype. The mice were serotyped for H-2 antigens by standard PVP hemagglutination techniques. Those mice that were homozygous for $H\text{-}2^b$ were excluded from the study as all $H\text{-}2^{b/b}$ mice are known to be resistant to CIA (2).

Results

Arthritis

F_1 Hybrids. 24 (B10 × SWR)F_1 mice were injected with bovine type II collagen, and four mice (17%) developed an atypical, mild, transient type of inflammatory arthritis (Fig. 1) after a latent period of over 2 mo (Table I). The swelling involved either the toes (Fig. 1) or the metatarsophangeal joints alone, but never the whole paw, and subsided spontaneously within ~1 mo. Neither of the parental strains, SWR ($n = 6$) nor B10 ($n = 10$), immunized simultaneously with BII in CFA, developed any signs of arthritis. This suggested that gene complementation between $H\text{-}2^q$ from SWR mice and non-H-2 genes (possibly the normal set of V_β TCR genes) from B10 mice might be occurring, which was responsible for the disease in these mice.

SWR Backcrosses. Of 18 mice in the SWR backcrosses immunized with BII, 6 (33%) developed arthritis that was again of a transient nature subsiding sponta-

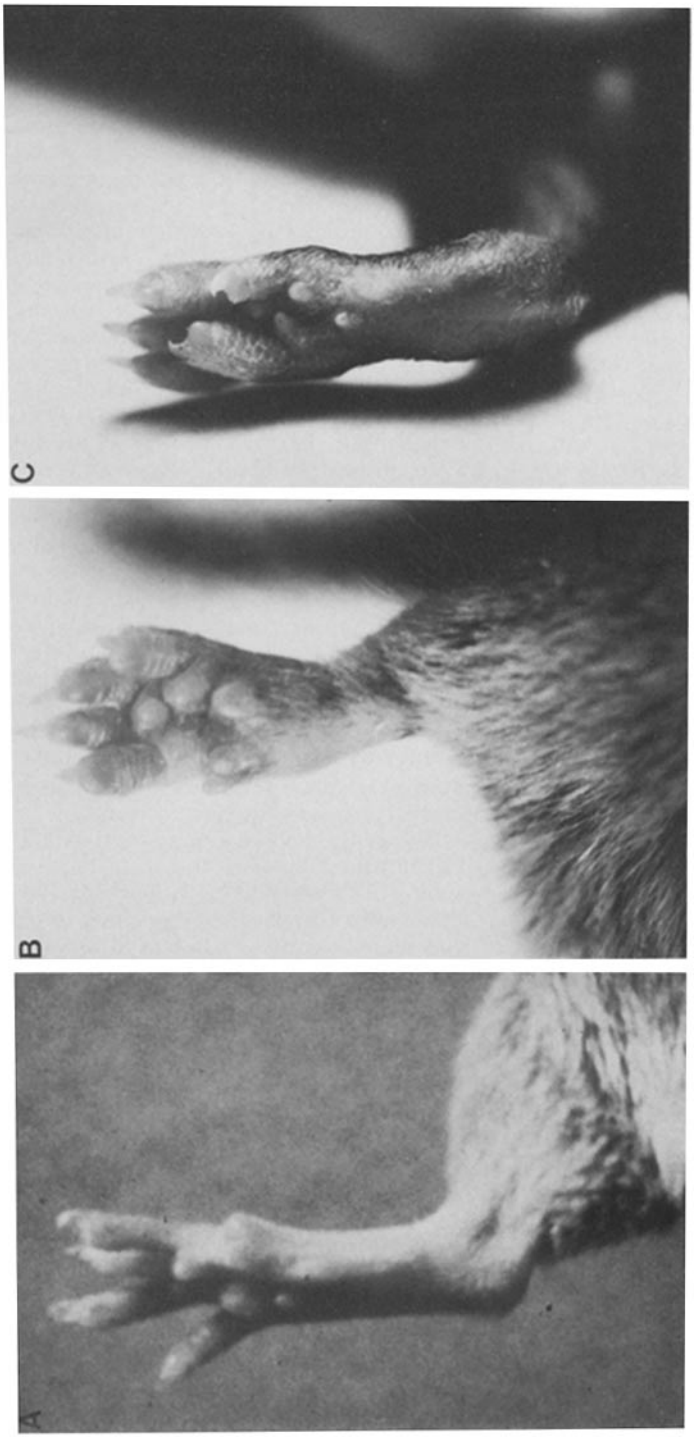

FIGURE 1. (*A*) Normal mouse hindpaw. (*B*) Arthritic front paw in an F_1 hybrid mouse with CIA. Note swelling in toes. (*C*) Deformed arthritic paw in a B10 backcross mouse with CIA.

TABLE I

Incidence and Severity of Arthritis in H-2^q–bearing Crosses between SWR and B10 Mice

	(B10 × SWR)F$_1$ hybrid	SWR backcross	B10 backcross	F$_2$ hybrid
Immunized mice				
Total number	24	18	25	25
Male/female	17:7	9:9	12:13	13:12
H-2$^{q/q}$/H-2$^{q/b}$	(all H-2$^{q/b}$)	7:11	(all H-2$^{q/b}$)	12:13
Arthritic mice				
Total number	4 (17%)	6 (33%)	17 (68%)	13 (52%)
Male/female	1:3	4:2	10:7	10:3
H-2$^{q/q}$/H-2$^{q/b}$	(all H-2$^{q/b}$)	1:5	(all H-2$^{q/b}$)	6:7
Transient arthritis				
Total number	4 (17%)	6 (33%)	10 (40%)	6 (24%)
Male/female	1:3	4:2	5:5	5:1
H-2$^{q/q}$/H-2$^{q/b}$	(all H-2$^{q/b}$)	1:5	(all H-2$^{q/b}$)	2:4
Day of onset*	76 ± 13	39 ± 4	57 ± 9	78 ± 11
Arthritic score‡	1 (1–2)	1 (1–2)	1 (1–2)	1 (all)
Severe arthritis				
Total number	0	0	7 (28%)	7 (28%)
Male/female	—	—	5:2	5:2
H-2$^{q/q}$/H-2$^{q/b}$	—	—	(all H-2$^{q/b}$)	4:3
Day of onset§	—	—	40 ± 6	49 ± 10
Arthritic score‡	—	—	6 (3–11)	3 (2–8)

* Mean ± SEM.

‡ Median (range given in parenthesis).

neously within ~1 mo as in the F$_1$ hybrids. However, the onset of arthritis was earlier in this group (Table I) and the arthritis was somewhat more severe, with the whole paw being involved in a few cases. Five of the six mice that developed arthritis were H-2$^{q/b}$, and only one was H-2$^{q/q}$. There were no obvious differences in the severity of arthritis between H-2$^{q/b}$ and H-2$^{q/q}$ mice in this group.

B10 Backcrosses. The B10 backcrosses, however, gave somewhat different results on immunization with BII. 7 of the 25 mice (28%) developed a chronic, deforming variety of arthritis (Fig. 1) described earlier in the literature (2, 10). 10 (40%) mice developed the atypical and transient form of CIA described above, with the onset being intermediate between that in the F$_1$ hybrids and SWR backcrosses. This indicated that genes in the B10 background could be responsible for the severe form of CIA in the B10 backcrosses, possibly due to a gene dose effect.

F$_2$ Hybrids. A similar picture as in the B10 backcrosses was seen in this group of mice. Seven mice (28%) developed the severe, deforming variety of arthritis, while six (24%) developed the transient and mild form. The onset and severity of the severe form was similar to that seen in the B10 backcrosses (Table I), and there were similar numbers of H-2$^{q/q}$ and H-2$^{q/b}$ mice among these mice. Routine histopathology of decalcified paraffin sections of a deformed paw (not shown) revealed extensive cartilage and bone erosions with infiltration of mononuclear cells and fibroblasts. The onset of the transient form was similar to that seen in the F$_1$ hybrids.

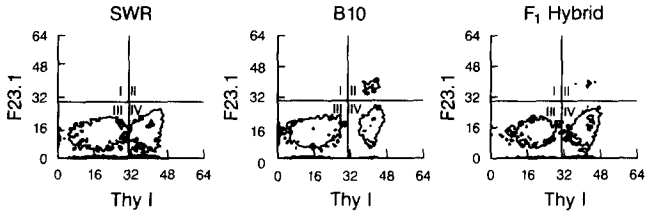

FIGURE 2. Dual fluorescence contours of PBLs from SWR, B10, and F_1 hybrid mice using anti-Thy 1 (green) and F23.1 (red) antibodies. Percentages of T cells with F23.1 phenotype are 0 for SWR, 18 for B10, and 10 for F_1 hybrid.

Anti–Bovine Type II Collagen Antibody

The serum anti–type II collagen antibody titers 28 d after immunization with BII were checked in some of the mice from each of the crosses (data not shown). Using Student's *t* test, no statistically significant difference ($p > 0.05$) was found in the anti-BII antibody titers (total IgG, IgG2a, IgG2b, IgG1) between the arthritic and nonarthritic mice in each of the crosses, though the titers in the arthritic mice tended to be more than the nonarthritic mice in the F_2 hybrid mice (total Ig, IgG2a, Ig2b) ($0.1 > p > 0.05$). There was also no difference in titers between mice with transient arthritis vs. mice with severe arthritis. Lack of correlation of anti-type II collagen antibody titers and disease has been observed earlier in various studies (2, 11).

Segregation of $V_\beta 8$ TCR Genes (F23.1 phenotype)

To study the association of TCR V_β genes with arthritis, the presence of the F23.1 T cell phenotype was checked by flow cytometry in mice with disease in the F_2 population. This antibody binds only to T cells using the $V_\beta 8$ family genes in their T cell receptors, as mentioned earlier. Since the T cell receptor genes are allelically excluded (14), mice heterozygous for the wild-type TCR had about one-half the number of $F23.1^+$ T cells as compared with those homozygous for the wild-type TCR (Fig. 2). Three mice were homozygous and eight were heterozygous for the F23.1 phenotype among the F_2 arthritic mice. All six of the $H\text{-}2^{q/q}$ homozygous mice in the F_2 generation with arthritis had circulating $F23.1^+$ T cells. However, two of seven $H\text{-}2^{q/b}$ heterozygous mice with arthritis did not have circulating $F23.1^+$ T cells.

Discussion

Arthritis has been induced in crosses between two CIA-resistant parents showing that a gene complementation had taken place. The $I\text{-}A^q$ gene is known to be a susceptibility gene from our previous studies (2, 6) and is contributed by SWR. The gene or genes contributed by B10 must also be one(s) involved in the immune response for arthritis to occur. F2 hybrids were selected for segregation analysis to study whether V_β TCR genes, present in the wild form in B10, could be the complementing susceptibility gene from the latter. Since even in B10.Q (which is the prototypic susceptible mouse strain) mice, the incidence of CIA is only ~75% (2) and never a complete 100%, segregation analysis for gene markers can only be done in mice that develop arthritis. 11 of the 13 mice in the F_2 generation with CIA had circulating $F23.1^+$ T cells. The two mice that lacked $F23.1^+$ T cells had an $H\text{-}2^{q/b}$ haplotype. One possibility that should be considered in these mice is the potential presentation of the arthritogenic epitope on collagen

by hybrid Ia molecules (15). Hybrid Ia molecules are known to be recognized by a unique set of T cells that do not recognize either of the partner Ia antigens by themselves (15). The pathogenic epitope thus could be presented by hybrid I-$A^{q/b}$ molecules to a set of T cells that use V_β TCR gene segments not deleted in SWR mice. However, a more likely possibility has emerged recently to explain the apparent paradox in the two "negative" mice. The tail DNA from the latter mice have since been probed with various V_β TCR probes by Southern blotting, and preliminary results (Haqqi, T. M., S. Banerjee, M. A. Behlke, D. Y. Loh, H. S. Luthra, C. S. David, manuscript in preparation) indicate that at least one B10-derived V_β TCR gene segment not present in SWR mice is present in both these mice. Thus, even these two F23.1$^-$ mice seem to have wild-type V_β TCR genes derived from B10.

SWR mice do show delayed type hypersensitivity (DTH) responses to native type II collagen after immunization with the latter (16). The T cell responses to the arthritogenic determinant(s) on type II collagen, however, may be defective. SWR mice thus could be resistant to CIA because they cannot develop arthritogenic T cells and T cell–dependent antibodies directed against epitopes on type II collagen in the joint, as this would require the usage of a particular set of V_β TCR genes that are missing in them. However, the presence or absence of another background gene in modulating arthritis cannot be ruled out since C_5 deficiency has been reported in SWR mice (17). The presence of C5 deficiency in both parents (NZB and SWR) does not, however, prevent the development of a severe prototypic immune complex disease (glomerulonephritis) in 100% of female F_1 hybrids in another disease model (18). Studies are underway to confirm the role of the TCR deletion in the resistance to CIA in SWR mice by studying susceptibility of the various crosses between SWR and C57L mice (H-2^b, with a TCR deletion exactly similar [reference 7] to SWR mice), and between SWR and A/Sn mice (C5 deficient). None of the mice in crosses from the first combination should develop arthritis, if the absent TCR genes do play a role in CIA resistance in SWR mice. On the other hand, induction of arthritis in the latter combination would suggest that C_5 deficiency is not a primary factor in the resistance to CIA in SWR mice.

Summary

Arthritis was induced by immunization of type II collagen in adjuvant in mice from H-2^q–bearing crosses between SWR (H-$2^{q/q}$) and B10 (H-$2^{b/b}$ mice), two strains known to be resistant to collagen-induced arthritis (CIA). The resistance of B10 is known to be due to its MHC haplotype, but it was postulated that the resistance of SWR mice which expresses the susceptible MHC haplotype could be due to the deletion of close to 50% of the V_β genes of the T cell receptor (TCR) in them. 17% of the F_1 hybrids, 33% of the SWR backcrosses, 68% of the B10 backcrosses, and 52% of the F_2 hybrids developed arthritis on follow-up to 5 mo after primary immunization with collagen. There was no significant difference in anti–type II collagen antibody titers between the arthritic and nonarthritic mice in each of these crosses. The segregation of the TCR genes with arthritis was determined in the F_2 population by typing with F23.1 mAb that reacts with T cells using $V_\beta 8$ subfamily genes in their TCRs. SWR mice are

F23.1$^-$ as $V_\beta 8$ genes are deleted in them. All six of arthritic mice homozygous for H-2^q, and thus with an H-2 haplotype similar to SWR mice, expressed the F23.1 marker. These studies indicate that for complete susceptibility to collagen-induced arthritis, not only is a susceptible MHC haplotype (H-2^q) important, but possibly also the presence of a subset of T cells using certain specific V_β genes in their TCRs. Other background genes may, however, modulate the severity of arthritis.

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