

Collagen-induced Arthritis in T Cell Receptor V_{β} Congenic B10.Q Mice

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Summary

B10.Q (H-2^q) mice congenic for the truncated T cell receptor (TCR) V_{β}^a and V_{β}^c haplotypes were derived to examine the influence of TCR V_{β} genomic deletions in murine collagen-induced arthritis (CIA). Previous studies using gene complementation and segregation analyses suggested that in SWR (H-2^q) mice, possession of the V_{β}^a gene deletion results in CIA resistance. However, other studies have suggested alternative hypotheses. Thus, analysis of TCR V_{β} congenic mice allows for direct examination of V_{β} genotypes in CIA control. After immunization with bovine type II collagen, B10.Q- V_{β}^a mice showed no difference in arthritis susceptibility, onset, or severity when compared with prototype B10.Q mice. In contrast, B10.Q- V_{β}^c mice, which lack the $V_{\beta}6$, 15, 17, and 19 families in addition to the V_{β}^a deletion, were highly resistant to CIA. In vivo depletion of $V_{\beta}6^+$ T cells in B10.Q- V_{β}^a mice significantly delayed arthritis onset suggesting that, among those V_{β} genes present in V_{β}^a but absent in V_{β}^c , $V_{\beta}6^+$ T cells contribute to arthritogenesis. Our findings show that, in B10.Q- V_{β} congenic mice, while the V_{β}^a genotype does not prevent CIA, the highly truncated V_{β}^c genotype renders B10.Q mice resistant to CIA. Thus, deletions within the V_{β} TCR genome can indeed influence CIA and suggests that the TCR repertoire displays only marginal flexibility in response to arthritogenic stimuli.

Collagen-induced arthritis (CIA)¹ in mice is an experimental animal model bearing similarities to human rheumatoid arthritis. CIA is typically induced by immunizing genetically susceptible mice with native type II collagen (CII) in CFA (1). Both humoral (2) and cellular (3) immunity against CII can be detected in this inflammatory polyarthritis. Although anti-CII antibodies contribute to the arthritogenic process (4), severe and chronic arthritis stems from activation of CII-reactive T cells (5, 6) responsible for the release and/or induction of proinflammatory cytokines such as IFN- γ , TNF- α , and IL-1 (7-10).

Previous studies established that susceptibility to CIA is associated with genes of the MHC, in particular gene products residing within the H-2A subregion (1). Mouse strains that bear the H-2^q or H-2^r haplotype can consistently develop CIA, whereas strains bearing other haplotypes such as H-2^b or H-2^d are highly resistant (1). However, among the H-2^q strains, SWR and AU/ssJ fail to develop CIA (11,

12). Likewise, RIII/J (H-2^r) mice are similarly CIA resistant (13). Work by Behlke et al. (14) showed that SWR mice were among four mouse strains that possess a deletion of ~50% of their V_{β} genome. A similar deletion was subsequently detected in the AU/ssJ strain (12). These strains have been designated as V_{β}^a haplotype and lack the members of the $V_{\beta}8$ and 5 gene families as well as the $V_{\beta}9$, 11, 12, and 13 gene segments (14). In addition, Haqqi et al. (15) showed that RIII/J animals also possess a highly truncated TCR V_{β} genotype composed of the genes deleted in V_{β}^a mice along with four additional V_{β} genes ($V_{\beta}6$, 15, 17, and 19). This unique deletion has been designated V_{β}^c and encompasses ~70% of the V_{β} genome.

To determine whether the TCR V_{β} deletion in SWR contributes to CIA resistance, gene complementation studies were undertaken. Analysis of F₂ progeny from a cross between the SWR strain and B10 mice, which bear the CIA-resistant H-2^b haplotype but possess the wild-type V_{β}^b genotype, revealed that these offspring were CIA susceptible and a strong correlation between arthritis susceptibility, possession of the V_{β}^b allele, and homozygosity for the H-2^q haplotype was observed (16). A dominant role for C5 deficiency

¹ Abbreviations used in this paper: CIA, collagen-induced arthritis; CII, native type II collagen.

contributing to CIA resistance in the SWR strain was eliminated by the observation that offspring of a cross between SWR mice and A/Sn animals (V_{β}^b , C5 deficient) remained susceptible to CIA (17). Taken together, these findings suggested that the V_{β}^a genotype may confer resistance to CIA in SWR and other H-2^q mice.

Interestingly, subsequent studies examining the role of the V_{β}^a deletion in CIA resistance have been controversial. Spinella et al. (18) and Andersson et al. (19) reported no correlation between CIA susceptibility and V_{β} haplotype in arthritic F₂ progeny of a DBA/1 × SWR cross. It is unclear whether genes provided by the DBA/1 genome, a strain prone to the development of spontaneous arthritis (20), contributed to these observations. Attempts to modulate arthritis induction in DBA/1 mice, by depleting T cells using V_{β} TCRs encoded within the V_{β}^a deletion, have led to conflicting results (21–23). Finally, Mori et al. (24) demonstrated that in the expression of a transgenic TCR β chain, derived from a CII-reactive arthritogenic T cell clone, mice failed to confer CIA susceptibility in SWR mice. However, expression of this transgene enhanced CIA development in (DBA/1 × SWR)_{F1} mice. This observation supports a role for TCR V_{β} genes in arthritogenesis. In addition, the varying effects of this V_{β} TCR transgene on CIA, in the context of different genetic backgrounds, underscored the multiple genetic control of this autoimmune disease.

Given the polygenic nature of CIA in mice, it is clear why previous gene complementation and transgenic studies have failed to unequivocally determine the influence of TCR V_{β} gene deletions on the induction and development of arthritis. To directly address this issue, it is necessary to derive strains of CIA susceptible mice congenic for the three known V_{β} haplotypes. Such an approach eliminates the contribution of background genes in the experimental outcome, a problem inherent in gene complementation analysis. Thus, we have developed lines of B10.Q (H-2^q) mice congenic for the V_{β}^a and V_{β}^c genotype. We report here that, in the B10.Q strain, possession of the V_{β}^a TCR gene deletion has no influence on the induction or development of CIA. However, B10.Q mice congenic for the V_{β}^c genotype were highly resistant to CIA. Our studies demonstrate that, in association with certain background genes, TCR V_{β} haplotypes can indeed play a major role in CIA development and illustrates the necessity of particular TCR V_{β} genes in the generation of arthritogenic responses against CII.

Materials and Methods

Mice. All mice used in this study were bred and maintained in the Immunogenetics Mouse Colony of the Mayo Clinic. Breeding of the TCR V_{β} congenic lines was achieved as detailed in Fig. 1. Mice of both sexes were used in this study and were 8–12 wk of age at the beginning of the experiment.

Induction of CIA. Native bovine CII, isolated and purified as previously described (1), was dissolved overnight at 4°C in 0.01 N acetic acid and then emulsified at a 1:1 ratio with CFA (*Mycobacterium tuberculosis* strain H37Ra; Difco Laboratories Inc., Detroit, MI). Mice were immunized with 100 μ l (containing 100 μ g bovine CII) of cold emulsion intradermally, at the base of the tail.

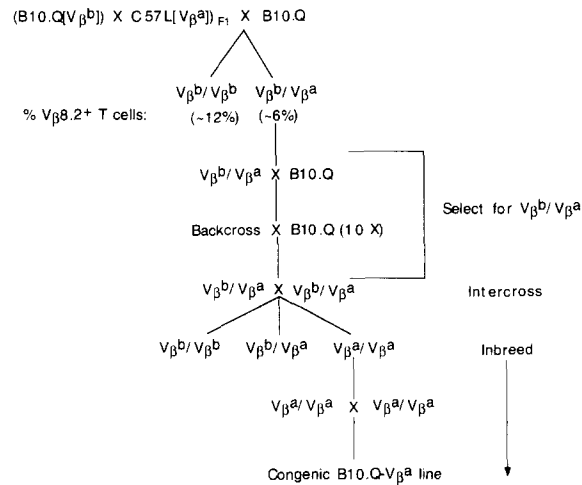


Figure 1. Diagrammatic illustration of the generation of congenic B10.Q- V_{β}^a mice. Segregation of the V_{β}^a allele was evaluated by expression of $V_{\beta}8.2$ TCR on PBL. The generation of B10.Q- V_{β}^c mice was achieved in a similar fashion using (RIII s/J[V β^c] × B10.Q)_{F1} mice in the original backcross with B10.Q.

All mice were monitored three to four times a week for CIA development over a 16-wk period. Arthritic severity was determined as detailed previously (1) using a scoring system for each paw as follows: 1, redness or swelling in paw or toes; 2, severe swelling and/or joint deformity; 3, joint ankylosis. The arthritic score per paw was summed to give a maximal possible score of 12 for each individual animal. The mean arthritic score/group was determined using arthritic mice only.

Flow Cytometric Analysis. Determination of normal V_{β} TCR expression in B10.Q, B10.Q- V_{β}^a , and B10.Q- V_{β}^c mice was achieved as follows: briefly, splenic T lymphocytes were purified by incubation over a nylon wool column using standard procedures (25). The nonadherent cells were washed extensively in phosphate-buffered saline containing 1% bovine serum albumin and 0.1% sodium azide. Approximately 10⁶ cells were then incubated with one of the following V_{β} TCR-specific mAbs: B20.6, rat anti- $V_{\beta}2$ (26); KT4, rat anti- $V_{\beta}4$ (27); MR9-8, mouse anti- $V_{\beta}5.1$ (28); MR9-4, mouse anti- $V_{\beta}5.1.2$ (29); 44-22-1, rat anti- $V_{\beta}6$ (30); TR310, rat anti- $V_{\beta}7$ (31); F23.1, mouse anti- $V_{\beta}8.1.2.3$ (32); KJ16, rat anti- $V_{\beta}8.1.2$ (33); F23.2, mouse anti- $V_{\beta}8.2$ (34); MR10-2, mouse anti- $V_{\beta}9$; RR3-15, rat anti- $V_{\beta}11$ (35); 14-2, rat anti- $V_{\beta}14$ (36); and KJ23a, mouse anti- $V_{\beta}17a$ (37). After a 30-min incubation at 4°C, the cells were washed then incubated with an FITC-conjugated Fab₂ fragment specific for either mouse IgG, rat IgG, or rat IgM (Accurate Chemical & Science Corp., Westbury, NY). After extensive washing, all samples received a 1:1 mixture of mAbs specific for mouse CD4 and CD8 (GIBCO BRL, Gaithersburg, MD) directly conjugated with phycoerythrin or RED 613, respectively. The cells were then incubated 30 min at 4°C, washed, and fixed with 1% formalin before analysis. To determine the level of circulating $V_{\beta}6$ T cells after in vivo treatment with the $V_{\beta}6$ -specific mAb 44-22-1, mice were bled via the tail artery and the lymphocyte population was isolated by centrifugation over a Ficoll-Hypaque gradient. After washing, the cells were treated as detailed above. The level of V_{β} TCR expression on peripheral CD4⁺ and CD8⁺ lymphocytes was determined by three-color fluorescence flow cytometry using FACS[®] Vantage flow cytometer (Becton Dickinson & Co., Mountain View, CA).

Statistical Analysis. The incidence of arthritis between groups

was determined using χ^2 analysis. The mean day of arthritis and arthritic severity between groups was determined using the non-parametric Mann-Whitney *U* test.

Results

Generation of B10.Q- V_{β} Congenic Mice. The goal of this study was to directly address the role of V_{β} TCR gene deletions in the induction and perpetuation of CIA. Thus, congenic mice of the B10.Q background were developed which possessed either the truncated V_{β}^a or V_{β}^c genotypes. Fig. 1 illustrates the breeding strategy employed to derive the V_{β} TCR congenic lines. Briefly, V_{β}^b -bearing B10.Q mice were mated with C57L (V_{β}^a) animals and the F_1 progeny were backcrossed to B10.Q. Offspring of the (B10.Q \times C57L) $_{F_1}$ \times B10.Q cross were typed for MHC haplotype using standard hemagglutination techniques, and V_{β} haplotype by flow cytometric analysis of PBL using the $V_{\beta}8.2$ -specific mAb F23.2. Mice that typed as H-2^{q/q}, $V_{\beta}^{a/b}$ were selected and backcrossed to the B10.Q strain for 10 generations. After the 10th backcross, $V_{\beta}^{a/b}$ offspring were intercrossed and the $V_{\beta}^{a/a}$ progeny selected to establish the congenic B10.Q- V_{β}^a line. Establishment of the B10.Q- V_{β}^c line was achieved using a similar strategy in which V_{β}^c -bearing RIII/J mice were initially mated with B10.Q mice and the $V_{\beta}^{b/c}$ offspring selected during backcross generations to B10.Q.

Analysis of Peripheral V_{β} TCR Repertoire in B10.Q- V_{β} Congenic Mice. Given the introduction of truncated V_{β} genotypes into B10.Q mice, the expression of V_{β} TCR on splenic T cells in B10.Q, B10.Q- V_{β}^a , and B10.Q- V_{β}^c animals was de-

termined. Using a panel of V_{β} TCR-specific mAb, flow cytometric analysis revealed that, in general, both B10.Q- V_{β}^a and B10.Q- V_{β}^c mice displayed an increase in expression of nondeleted V_{β} TCR (Table 1). Interestingly, introduction of the functional $V_{\beta}17a$ gene in B10.Q- V_{β}^a mice led to a high expression of $V_{\beta}17^+$ T cells in the CD4 subset. No difference was observed in the CD4/CD8 ratio between the B10.Q, B10.Q- V_{β}^a , and B10.Q- V_{β}^c strains (data not shown). In both the B10.Q- V_{β}^a and B10.Q- V_{β}^c strains, the panel of V_{β} TCR-specific mAbs used for analysis accounted for ~ 38 and 21% of the total CD4⁺ and CD8⁺ T cell populations, respectively. In contrast, 82 and 67% of the V_{β} -bearing CD4⁺ and CD8⁺ T cells could be accounted for in B10.Q mice. Therefore, a majority of V_{β} TCR genes that were not quantitated by flow cytometry (V_{β} 1, 3, 10, 15, 16, 18, 19, and 20) appear to be used by the congenic B10.Q- V_{β}^a and B10.Q- V_{β}^c mice to shape their respective peripheral TCR repertoires.

CIA Susceptibility in B10.Q- V_{β}^a Mice. To determine the influence of the V_{β}^a haplotype in H-2^q-restricted CIA, the susceptibility of congenic B10.Q- V_{β}^a mice to arthritis induction was evaluated. Fig. 2 shows that, after immunization with bovine CII, B10.Q- V_{β}^a mice remained susceptible to CIA. Analysis of 23 animals demonstrated that the B10.Q- V_{β}^a strain displayed no noticeable difference in susceptibility, disease onset, or arthritic severity when compared with B10.Q mice. Thus, in B10.Q mice, possession of the V_{β}^a genotype does not affect the induction or development of CIA.

Arthritis Susceptibility in B10.Q- V_{β}^c Mice. To test the effect

Table 1. Peripheral Expression of V_{β} T Cell Receptors in B10.Q, B10.Q- V_{β}^a , and B10.Q- V_{β}^c Mice*

| V_{β} | Percentage of positive T cells (mean \pm SD) | | | | | |
|--------------------|--|----------------|----------------------|---------------|----------------------|---------------|
| | B10.Q | | B10.Q- V_{β}^a | | B10.Q- V_{β}^c | |
| | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 |
| $V_{\beta}2$ | 2.3 \pm 0.3 | 0.3 \pm 0.1 | 3.9 \pm 2.6 | 0.9 \pm 1.0 | 9.9 \pm 0.8 | 3.0 \pm 2.0 |
| $V_{\beta}4$ | 6.2 \pm 0.5 | 2.8 \pm 0.5 | 8.8 \pm 1.2 | 6.1 \pm 0.3 | 14.8 \pm 1.8 | 8.3 \pm 1.3 |
| $V_{\beta}5.1.2$ | 4.8 \pm 0.5 | 15.6 \pm 0.2 | Deleted | Deleted | Deleted | Deleted |
| $V_{\beta}5.1$ | 2.7 \pm 0.3 | 6.6 \pm 1.1 | Deleted | Deleted | Deleted | Deleted |
| $V_{\beta}6$ | 2.9 \pm 1.0 | 5.1 \pm 0.6 | 5.4 \pm 0.9 | 5.8 \pm 1.9 | Deleted | Deleted |
| $V_{\beta}7$ | 1.6 \pm 0.1 | 3.3 \pm 0.3 | 2.3 \pm 0.2 | 4.8 \pm 0.5 | 2.5 \pm 0.7 | 6.8 \pm 2.1 |
| $V_{\beta}8.1.2.3$ | 21.8 \pm 3.7 | 13.5 \pm 2.7 | Deleted | Deleted | Deleted | Deleted |
| $V_{\beta}8.1.2$ | 12.8 \pm 1.2 | 3.5 \pm 1.1 | Deleted | Deleted | Deleted | Deleted |
| $V_{\beta}8.2$ | 13.4 \pm 0.4 | 7.6 \pm 0.4 | Deleted | Deleted | Deleted | Deleted |
| $V_{\beta}9$ | 1.6 \pm 0.6 | 3.1 \pm 0.9 | Deleted | Deleted | Deleted | Deleted |
| $V_{\beta}11$ | 5.3 \pm 0.5 | 3.4 \pm 0.6 | Deleted | Deleted | Deleted | Deleted |
| $V_{\beta}14$ | 7.0 \pm 3.3 | 1.9 \pm 2.4 | 6.3 \pm 1.5 | 1.5 \pm 0.6 | 8.7 \pm 1.8 | 3.0 \pm 0.5 |
| $V_{\beta}17$ | Nonfunctional | Nonfunctional | 12.9 \pm 0.9 | 2.2 \pm 0.4 | Deleted | Deleted |

* Normal B10.Q, B10.Q- V_{β}^a , and B10.Q- V_{β}^c mice were killed and their splenic T lymphocytes isolated and stained for V_{β} TCR expression as detailed in Materials and Methods. Mean values \pm SD were determined based on three experiments.

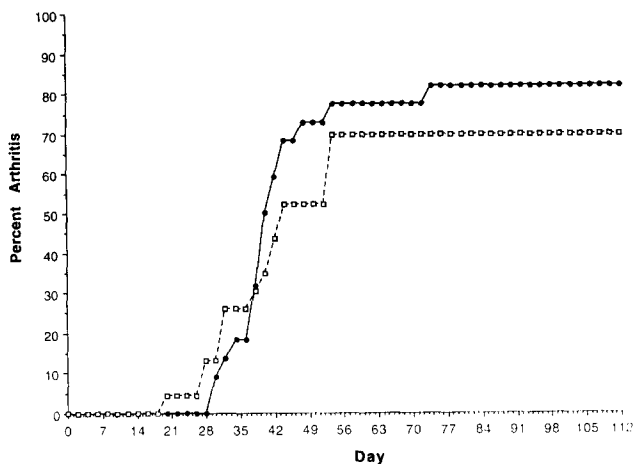


Figure 2. Development of CIA in congenic B10.Q- $V\beta^2$ mice. B10.Q (solid line, $n = 23$) and B10.Q- $V\beta^2$ mice (broken line, $n = 23$) were immunized with 100 μg bovine CII in CFA on day 0 and monitored regularly for the development of CIA. No difference in CIA severity was observed between the two groups.

of a highly truncated $V\beta$ TCR genome on H-2^q-restricted CIA, the susceptibility of B10.Q- $V\beta^c$ mice to arthritis induction was also determined. In contrast to the B10.Q- $V\beta^2$ strain, B10.Q- $V\beta^c$ mice were markedly resistant to CIA (Fig. 3). After immunization with bovine CII, only 4 of 13 animals developed arthritis over the 16-wk observation period, with 2 of the 4 arthritic mice displaying a transient disease. In addition, Fig. 4 illustrates that, of the B10.Q- $V\beta^c$ mice that developed CIA, arthritic severity was mild compared with B10.Q animals. These findings show that the highly truncated $V\beta^c$ genotype can dramatically alter arthritis susceptibility in B10.Q mice.

Modulation of CIA Development in B10.Q- $V\beta^2$ Mice Upon In Vivo Depletion of $V\beta 6^+$ T Cells. The susceptibility of B10.Q- $V\beta^2$ mice and resistance of B10.Q- $V\beta^c$ animals to CIA

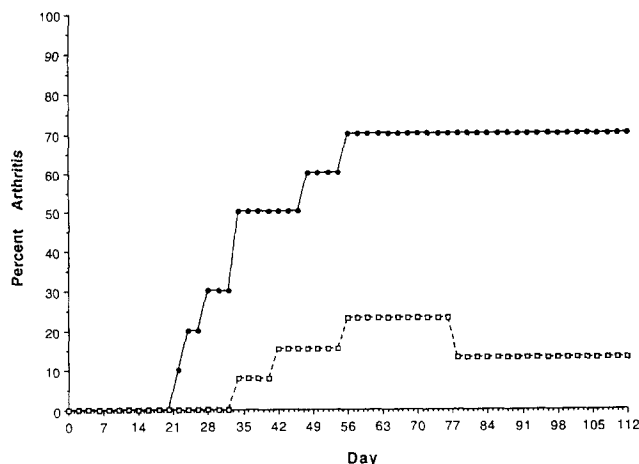


Figure 3. Resistance of B10.Q- $V\beta^c$ mice to CIA after immunization with bovine CII. B10.Q (solid line, $n = 10$) and B10.Q- $V\beta^c$ mice (broken line, $n = 13$) were immunized with 100 μg bovine CII in CFA and monitored regularly for the development of CIA.

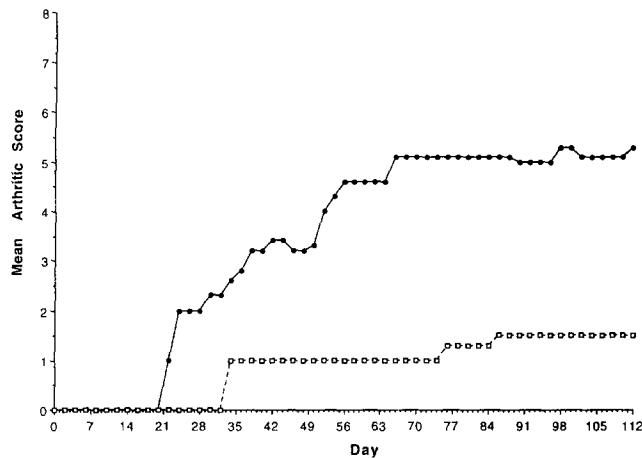


Figure 4. Progression of arthritis in B10.Q and B10.Q- $V\beta^c$ mice. Arthritic B10.Q (solid line) and B10.Q- $V\beta^c$ (broken line) mice from Fig. 3 were monitored for the progression of clinical arthritis. The mean arthritic severity in B10.Q- $V\beta^c$ mice was significantly lower ($p < 0.01$) vs. B10.Q animals throughout the experiment.

suggested that $V\beta$ genes present in the $V\beta^2$ genotype but absent in $V\beta^c$ ($V\beta 6, 15, 17,$ and 19) may play a role in arthritogenesis. Previous work by Anderson et al. (38) showed that the clonal elimination of Mls-1^a reactive T cells ($V\beta 6, 7, 8.1,$ and 9), by mating B10.Q mice with CIA resistant BALB.D2.Mls-1^a congenic animals, could lead to a decreased incidence of CIA in the F₁ progeny. Moreover, PCR analysis of $V\beta$ mRNA transcripts from arthritic joints of CII-immunized B10.Q mice revealed a predominance of $V\beta 8.2$ and $V\beta 6$ TCRs (39). Together, these three findings strongly suggested that in the B10.Q strain, $V\beta 6$ -bearing T cells may play a role in the induction and/or progression of CIA.

To determine the contribution of $V\beta 6^+$ T cells in CIA, the effect of depleting $V\beta 6$ -bearing T cells in vivo on arthritis induction in B10.Q- $V\beta^2$ mice was examined. As seen in Fig. 5, administration of the $V\beta 6$ -specific mAb 44-22-1 to B10.Q- $V\beta^2$ mice 3 d before immunization with bovine CII significantly delayed the development of CIA when compared with control animals treated with PBS (mean day of onset: 64 d vs. 42 d, $p = 0.019$). Analysis of PBL showed a marked reduction of $V\beta 6^+$ T cells in both the CD4 and CD8 subsets of anti- $V\beta 6$ -treated mice for up to 28 d with detectable levels of $V\beta 6^+$ T cells measured by 42 d (Fig. 6). Therefore, these data show that the early depletion of $V\beta 6^+$ T cells can modulate arthritogenesis in B10.Q- $V\beta^2$ mice. In addition, these findings implicate a role for $V\beta 6$ -bearing T cells in the recognition of arthritogenic epitopes on CII and the eventual induction of CIA.

Discussion

It is known that susceptibility to CIA in mice is dependent upon possession of the appropriate MHC haplotype (1) as well as CII specific arthritogenic T cells (5, 6). The T cell dependence of this disease has led to the suggestion that the population of T cells specific for CII may use a limited repertoire of TCR $V\alpha$ and $V\beta$ genes. Indeed, a concerted effort

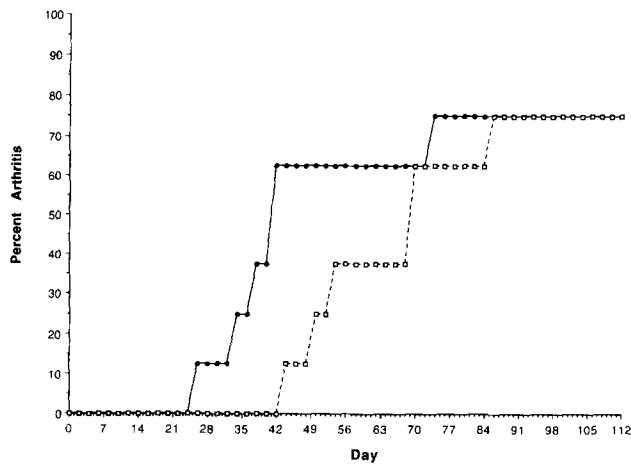


Figure 5. Delay of CIA development in B10.Q- V_{β}^2 mice after in vivo depletion of $V_{\beta}6^+$ T cells. B10.Q- V_{β}^2 mice were given an injection of 100 μg i.v. of the anti- $V_{\beta}6$ mAb 44-22-1 (broken line, $n = 8$) or 100 μl of PBS (solid line, $n = 8$) on day -3. On day 0, both groups were immunized with 100 μg bovine CII in CFA. The day of CIA onset was significantly delayed in anti- $V_{\beta}6$ -treated mice compared with controls (mean day of onset \pm SEM: anti- $V_{\beta}6 = 64.3 \pm 10.4$, PBS = 42.2 ± 6.5 , $p = 0.019$).

to test this hypothesis has been put forth by numerous investigators. However, unequivocal demonstration of a restricted TCR repertoire in CIA has remained elusive. Clearly, the polygenic nature of CIA, as well as species specific differences in the arthritogenicity of CII (40), have hampered attempts at reconciling experimental discrepancies among various laboratories. Such discrepancies are best depicted in experiments devoted to examining the role of the V_{β}^2 gene deletion in CIA (16-19). Initial studies by Banerjee et al. (16), using progeny of a B10 \times SWR cross, suggested that possession of the V_{β}^2 genotype results in CIA resistance. In contrast, studies by Spinella et al. (18) and Andersson et al. (19), using DBA/1 \times SWR crosses, found no correlation between the V_{β}^2 haplotype and CIA resistance. Therefore, it was the goal of this study to develop TCR V_{β} congenic B10.Q mice in an effort to directly examine the role of V_{β} haplotypes on CIA. Our data show that in the B10.Q strain, possession

of the V_{β}^2 genotype has no influence on CIA susceptibility. However, the arthritogenic TCR V_{β} repertoire displays only a limited degree of flexibility since introduction of the severely truncated V_{β}^c genotype renders B10.Q mice resistant to CIA.

Development of TCR V_{β} congenic mice allows one the opportunity to not only examine the influence of such gene deletions in autoimmune diseases, but also a chance to explore the effect of V_{β} haplotypes on TCR repertoire development in a fixed genetic background. In the B10.Q background, both congenic V_{β}^2 and V_{β}^c animals displayed a generalized increase in the expression of nondeleted V_{β} gene families with B10.Q- V_{β}^c mice expressing the most dramatic enhancement. Analysis of TCR V_{β} congenic B10 mice which possessed either the H-2^{b,k,r} or ^s haplotypes yielded similar results (Nabozny, G. H., unpublished observations). Moreover, the observation that approximately one third of the V_{β}^+ T cells were accounted for by flow cytometry in B10.Q- V_{β}^2 and B10.Q- V_{β}^c mice implicates a major contribution of the V_{β} 1, 3, 10, 15, 16, 18, 19, and 20 TCR genes in the peripheral repertoire of these mice.

Given the current discrepancies surrounding the role of the V_{β}^2 genotype in CIA resistance in SWR (H-2^a) mice, it was of great interest to determine the susceptibility of B10.Q- V_{β}^2 mice to CIA. After immunization with bovine CII, B10.Q- V_{β}^2 mice displayed no difference in arthritis susceptibility, onset or severity when compared with prototype B10.Q animals (Fig. 2). This finding clearly shows that in B10.Q mice, possession of the V_{β}^2 genotype alone is insufficient to cause CIA resistance.

Previous studies from our laboratory demonstrated that offspring of a (C57L[V_{β}^2] \times SWR)_{F1} \times C57L cross failed to develop CIA (17), thereby further implicating the V_{β}^2 genotype in CIA resistance. In light of our current findings in B10.Q- V_{β}^2 mice, it is clear that additional genetic factors, possibly in combination with the V_{β}^2 haplotype, render some H-2^a animals CIA resistant. It is possible that C57L mice share an unidentified genetic lesion with the SWR strain that contributed to the previous observations. Alternatively, the presence of the heterozygous H-2^{b/a} haplotype in (C57L \times SWR)_{F1} \times C57L animals may have led to the presenta-

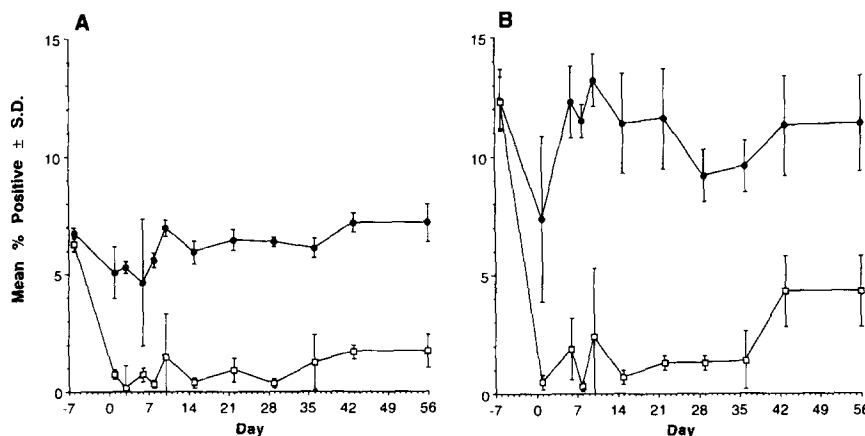


Figure 6. Level of $V_{\beta}6^+$ T cells in B10.Q- V_{β}^2 mice after administration of anti- $V_{\beta}6$ mAb. B10.Q- V_{β}^2 mice were injected with 100 μg anti- $V_{\beta}6$ mAb (open squares) or 100 μl PBS (solid circle) i.v. on day 0. The levels of circulating CD4^+ (A) and CD8^+ (B) $V_{\beta}6$ -bearing T cells in PBL was determined by flow cytometric analysis as described in Materials and Methods. The mean percent \pm SD was calculated based on three to six mice/time point.

tion of a low frequency of arthritogenic CII epitopes in association with I-A^q due to competition with I-A^b, a CIA nonsusceptible haplotype (1). This decrease, together with the V β^a genotype, may have resulted in suboptimal triggering of arthritogenic T cells. The incidence of CIA in (B10-V β^a \times B10.Q-V β^a)_{F1} mice is currently under analysis to address this hypothesis.

Although the B10.Q-V β^a strain displayed a similar pattern of arthritis susceptibility to B10.Q mice, one cannot say a priori that the mechanisms underlying the arthritogenic response is identical in both strains. To date, two H-2^q-restricted T cell epitopes on the CII molecule, residues 181-209 and 260-270, have been identified which may play a role in CIA (41, 42). It is possible that B10.Q-V β^a T cells respond to only one of the two determinants, but, this response may still be sufficient to induce arthritis (Fig. 7). A second possibility is that introduction of the V β^a genotype may lead to the recognition of a distinct determinant within the immunodominant peptide, as previously shown for H-2^d-restricted responses against the 110-121 peptide of sperm whale myoglobin (43-45). Finally, it is also possible that the genetic backgrounds in the H-2^q-bearing SWR, DBA/1, and B10.Q strains generate different self peptides that may modulate and/or influence potential CII-reactive T cells. Further studies are required to delineate the nature of CII-specific T cell responses in B10.Q-V β^a mice.

In contrast to the V β^a haplotype, introduction of the highly truncated V β^c genotype in B10.Q mice resulted in a marked resistance to CIA (Fig. 3). The differential susceptibility between the B10.Q-V β^a and B10.Q-V β^c strains pro-

vided an opportunity to map candidate V β TCR genes involved in the arthritogenic response. In vivo depletion of V $\beta 6^+$ T cells in B10.Q-V β^a animals led to significant delay in arthritis onset compared with control mice (Fig. 5). Thus, in the B10.Q background, V $\beta 6^+$ T cells participate in the induction of CIA. This finding is indirectly supported by two lines of experimental evidence. First, offspring of a (B10.Q \times BALB.D2.mls^s)_{F1} cross, which lack V $\beta 6^+$ T cells due to a Mls-1^s-mediated clonal deletion, displayed a reduced incidence of CIA (38). Second, Haqqi et al. (39) demonstrated a retention of V $\beta 6$ mRNA transcripts arthritic joints of B10.Q mice. Molecular analysis of V $\beta 6$ alleles between V β^a - and V β^b -haplotype mice revealed only minor differences (46) suggesting that our findings using B10.Q-V β^a mice are compatible with the aforementioned observations in V β^b strains.

The demonstration that V $\beta 6^+$ T cells contribute to CIA in B10.Q animals is in contrast to previous studies using another H-2^q strain, DBA/1. Chiochia et al. (21), Hom et al. (22), and Goldschmidt et al. (23), reported no effect on CIA induction in DBA/1 mice after the in vivo elimination of V $\beta 6^+$ T cells. However, analysis of CII-reactive T cell hybridoma clones from DBA/1 mice immunized with CII revealed a predominant usage of V $\beta 1^+$, V $\beta 8.2^+$, and V $\beta 6^+$ TCRs (47). On balance, our findings, in association with the previous studies, illustrate that a multiplicity of factors are responsible for shaping the arthritogenic TCR repertoire. Although our experimental approach using congenic mice precludes identification of such putative factors, our data does shed light on understanding the influence of V β TCR genotypes in CIA. Yet, one must note that even a small propor-

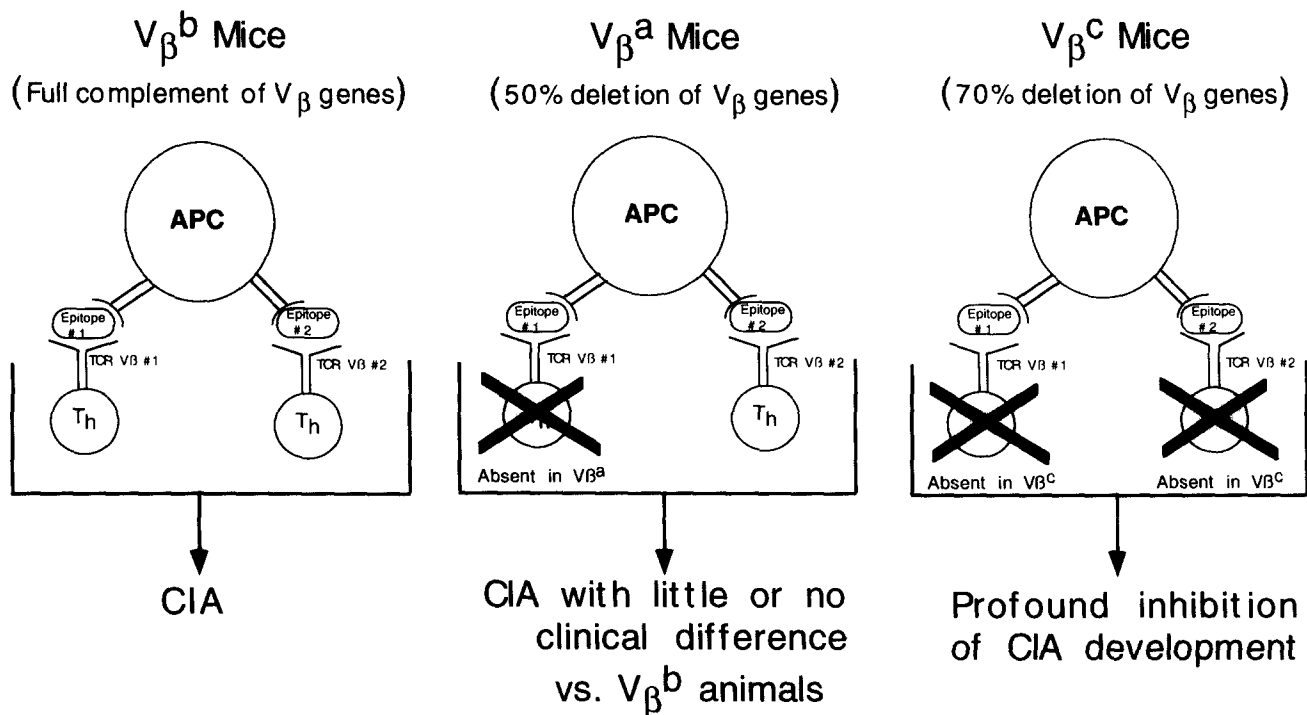


Figure 7. Possible mechanism for CIA development in B10.Q-V β congenic mice. Epitopes nos. 1 and 2 correspond to the H-2^q-restricted T cell determinants 181-209 (41) and 260-270 (42) on the CII molecule, respectively.

tion of B10.Q-V β^c mice still retained the ability to develop arthritis. Thus, identifying those factors which act in concert with TCR V β genes requires thorough investigation. Previous studies suggest that candidate factors may include species specific differences in CII arthritogenicity (40) and TCR V α genotypes (48). Additionally, the identification of non-MHC, non-TCR genetic influences in CIA, as previously reported for the nonobese diabetic mouse model of type I diabetes (49-51) remains to be determined. Clearly, elucidation of these influences is needed for a complete understanding of the mechanisms responsible for arthritogenesis.

In closing, this study is the first attempt to directly ad-

dress the influence of TCR V β genotypes on murine CIA. The use of TCR V β congenic mice not only allows a means to test the role of these genotypes in the generation of arthritis, but also examines the contribution of truncated V β genomes in the formation of the arthritogenic TCR repertoire. Moreover, the development of TCR V β congenic strains on a variety of H-2 haplotypes will enable investigators to address similar questions in other models of autoimmune disease. Answers to such issues may lead to a better understanding of the mechanisms underlying the development and triggering of autoimmune responses.

These studies were supported by National Institutes of Health grant AR-30752 and a grant from the Minnesota chapter of the Arthritis Foundation. Dr. Nabozny is supported by a Postdoctoral Fellowship from the Arthritis Foundation.

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Received for publication 19 January 1994 and in revised form 19 April 1994.

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