EXTENDED REPORT

IFN γ deficient C57BL/6 (H-2^b) mice develop collagen induced arthritis with predominant usage of T cell receptor V β 6 and V β 8 in arthritic joints

C-Q Chu, Z Song, L Mayton, B Wu, P H Wooley

Ann Rheum Dis 2003;62:983–990

See end of article for authors' affiliations

Correspondence to:
Dr P H Wooley,
Department of Orthopaedic
Surgery, Wayne State
University, 1 South, Hutzel
Hospital, 4707 St Antoine
Blvd, Detroit, MI 48201,
USA; ad8754@wayne.edu

Accepted 5 March 2003

Background: Transgenic deficiency in interferon γ (IFN γ) or IFN γ receptor makes resistant strains of mice bearing H-2^b or H-2^d susceptible to collagen induced arthritis (CIA).

Objective: To determine whether the escape from regulation of disease susceptibility at the major histocompatibility complex level involves a new use of autoimmune T cells expressing T cell receptor (TCR) V β that vary from the cell populations previously identified within arthritic joints.

Methods: Arthritis was induced by a standard protocol with type II bovine collagen (CII) in complete Freund's adjuvant. Clinical features, histopathology, immunological responses, and TCR profile in arthritic joints in IFN γ knockout C57BL/6 (B6.IFN γ KO) mice (H-2^b) were compared directly with those in DBA/1 mice (H-2^d).

Results: 60–80% of B6.IFN γ KO mice developed a progressive arthritis with a similar clinical course to classical CIA in DBA/1 mice. The affected joints in B6.IFN γ KO mice had an erosive form of arthritis with similar features to joint disease in DBA/1 mice. B6.IFN γ KO mice produced significantly higher levels of IgG2b and IgG1 autoantibodies to murine CII and showed increased proliferative response to CII compared with B6 mice. Comparable levels of interleukin 1 β and tumour necrosis factor α expression were detected in arthritic joints from B6.IFN γ KO and DBA/1 mice. B6.IFN γ KO mice used predominantly TCR V β 6 and V β 8 in arthritic joints. This TCR V β profile is similar to that found in DBA/1 mice with CIA.

Conclusions: C57BL/6 mice deficient in IFN γ production can develop arthritis that resembles classical CIA. These data suggest that IFN γ is a key factor mediating susceptibility to CIA.

Collagen induced arthritis (CIA) is a useful model for understanding the pathogenesis of rheumatoid arthritis. One prominent feature of CIA is that susceptibility to disease in mice is restricted to strains bearing major histocompatibility complex (MHC) class II H-2^a or H-2^e haplotype, while strains bearing other H-2 haplotypes are resistant to disease induction.¹ Subsequent studies have demonstrated the importance of CD4⁺ T helper (Th) cells, in particular Th1 subset, in CIA initiation and disease perpetuation, and the involvement of restricted T cell subsets in infiltration of the arthritic joint.^{2–6} Current dogma concerning the pathogenesis of CIA suggests that limited epitopes on collagen type II (CII) molecule are presented in association with H-2^a or H-2^e class II MHC molecules to “autoimmune” T cell subsets expressing predominantly V β 6 and V β 8.1 T cell receptors (TCRs), which are regulated through the expression of minor lymphocyte stimulating antigens.^{7–8} Disease induction is also dependent upon the production of antibodies reactive with autologous collagen,⁹ and the subsequent expression of disease may be modulated through cytokine expression, both by increase in the inflammatory response and the differentiation of Th1/Th2 reactivity. The role of interferon γ (IFN γ) in the development of CIA has been a focus of several studies. IFN γ has been used as a signature cytokine for Th1 cells because it is produced mainly by activated CD4⁺ T cells and NK cells. Theoretically, IFN γ is believed to be pathogenic in CIA and there is evidence supporting this notion. Cooper *et al* showed that IFN γ administration begun at the time of immunisation increased the incidence of arthritis and accelerated disease onset,¹⁰ whereas Boissier *et al* found high doses of IFN γ increased disease severity in arthritic mice.¹¹ However, other studies have suggested a protective role of IFN γ in CIA. Treatment of CIA with anti-IFN γ antibodies increased limb involvement in mice, although anti-

IFN γ antibodies did not significantly affect the incidence of arthritis, time of onset, degree of oedema, histopathological severity, or level of anti-collagen II (anti-CII) IgG antibodies.¹² The treatment of γ irradiated DBA/1 mice with IFN γ also inhibited adoptive arthritis upon transfer with CII immunised spleen cells.¹³ Recent studies using IFN γ receptor knockout (KO) mice on the CIA susceptible DBA/1 background suggested a beneficial role of IFN γ in this experimental disease. IFN γ receptor deficient DBA/1 mice developed more severe CIA with accelerated onset of disease than wild-type DBA/1 mice.^{14–15} These results prompted us to examine whether deletion of the IFN γ gene might break the resistance to CIA induction in strains of mice bearing other H-2 haplotypes than H-2^a or H-2^e. Ortmann and Shevach recently reported that arthritis could be induced in IFN γ deficient C57BL/6 (H-2^b), IFN γ deficient BALB/c (H-2^d), or IFN γ receptor deficient 129/Sv (H-2^b) mice, whereas the wild-type strains remained resistant.¹⁶ In our report we use classical CIA in DBA/1 mice for direct comparison to characterise arthritis in IFN γ KO C57BL/6 (B6.IFN γ KO) mice, and examine whether the escape from regulation of disease susceptibility at the class II MHC level involves a new use of autoimmune T cells expressing TCR V β that vary from the cell populations previously identified within the synovial infiltrate.

Abbreviations: CFA, complete Freund's adjuvant; CIA, collagen induced arthritis; CII, collagen type II; IFN γ , interferon γ ; IL1 β , interleukin 1 β ; KO, knockout; MHC, major histocompatibility complex; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; PBS, phosphate buffered saline; RT-PCR, reverse transcriptase-polymerase chain reaction; TCR, T cell receptor; Th, T helper; TNF α , tumour necrosis factor α .

MATERIALS AND METHODS

Mice

Female DBA/1, B6.129S7-*ifng*^{tm1ts} (B6.IF γ KO), and C57BL/6 (B6) mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and quarantined within our facility before experimentation. All mice were used at 8–10 weeks of age, and all procedures were approved by the Animal Investigation Committee, Wayne State University.

Collagens

Native bovine CII purified from bovine articular cartilage was provided by Dr Marie Griffiths, University of Utah. This bovine CII is free of other collagen types, proteoglycan, and pepsin. Native murine CII (free of collagen type I and containing <0.4% proteoglycan) was purchased from Elastin Products (Owensville, Missouri).

Induction of CIA

Native bovine CII was solubilised in 0.1 M acetic acid at 2 mg/ml and emulsified in equal volume of complete Freund's adjuvant (CFA) (Difco Laboratory, Detroit, MI) containing 5 mg/ml of killed *Mycobacterium tuberculosis* (H37Ra). Mice were immunised with 100 μ g bovine CII in CFA injected intradermally. For control experiments, mice were injected with 0.1 M acetic acid in CFA alone.

Clinical assessment of arthritis

All mice were examined daily for the initial visual appearance of arthritis for 65 days after immunisation. Arthritis of each individual limb of a mouse was graded using a scoring system described previously¹: 0, no visual arthritis; 1, erythema and swelling; 2, visible joint distortion; and 3, ankylosis of joint. The maximum score of a mouse is 12. Paw thickness was measured with a constant tension calliper three times a week.

Histological evaluation of arthritis

Sections of mouse limbs were stained with haematoxylin and eosin. The histological severity of arthritis was evaluated by assessing synovitis, pannus formation, and marginal erosion of the joint and articular architectural changes. Each component was graded from 0 to 5. The overall score reflects: 0, normal joint appearance; 1, minor changes, consistent with remission, may be clinically normal; 2, moderate inflammatory disease; 3, major inflammatory disease; 4, destructive, erosive arthritis; 5, destructive, erosive arthritis with major bone remodelling.

Anti-CII antibody quantification

Mouse serum samples were collected at various times. Antibodies of different isotypes to bovine and murine CII were determined by enzyme linked immunosorbent assay (ELISA), as previously described.¹⁷ Microtitre plates were coated with 10 μ g/well of native bovine or murine CII in potassium phosphate coating buffer (pH 7.4) overnight at 4°C, followed by blocking with a 16 hour incubation at 4°C with 5% fat-free dry milk in phosphate buffered saline (PBS, pH 7.4). Serum samples diluted at 1:1000 in PBS containing 0.5% Tween 20 and 2% fat-free dry milk were incubated overnight at 4°C. Goat antimouse IgG, IgG1, IgG2a, IgG2b, IgG3, IgM, IgA, and IgE conjugated to alkaline phosphatase (Southern Biotechnology, Birmingham, AL) diluted at 1:5000 were incubated for one hour at room temperature to detect anti-CII antibody isotypes and IgG subclasses. Sigma 104 PNPP substrate dissolved in glycine buffer (pH 10.4) was used to visualise the reaction. The optical density at 405 nm was read with a Molecular Devices plate reader (Molecular Devices, Menlo Park, CA). Antibody levels were expressed as arbitrary units with reference to a standard serum.

Cell proliferation assay

Draining lymph nodes were harvested 10 days after immunisation. Cell proliferation was performed by a non-radioactive method.¹⁸ Lymphocytes were incubated in T cell medium (RPMI 1640 with 7.5% fetal calf serum, 0.01 M HEPES, 5×10^{-5} M 2-mercaptoethanol, 2 mM L-glutamine) in the presence of native bovine CII (10 and 100 μ g/ml) diluted in medium, concanavalin A (3 μ g/ml), or medium alone. The cells were plated at 4×10^5 /well in triplicate in 96 well plates for 24 hours, and 10 μ l of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) solution (20 μ g/ml, Sigma) was added in the culture and incubated for a further 16 hours. MTT is a substrate for various dehydrogenase enzymes, and is modified to a coloured formazan product by living cells, but not dead cells. This conversion of MTT to a blue crystal has been adapted as measure of cellular activation and proliferation. The culture supernatant was replaced with 200 μ l of 10% sodium dodecyl sulphate solution and the plates were incubated at 37°C to facilitate crystal solubilisation. The optical density of the solution was read at 590 nm with a microplate photospectrometer (Molecular Devices).

Real-time reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was extracted from arthritic limbs with Trizol and chloroform according to the manufacturer's instruction (Invitrogen, Carlsbad, CA). cDNA was reverse transcribed from 0.5 μ g total RNA in a 40 μ l reaction mixture containing 500 μ mol/l each of deoxynucleotide triphosphates, 0.4 U/ μ l RNAase inhibitor, 2.5 μ M of reverse transcriptase (Gibco-BRL). The reaction mixture was incubated in a DNA thermal cycler (Perkin Elmer, CT) at 25°C for 10 minutes, 48°C for five minutes, and then 95°C for five minutes. Real-time PCR was performed according to the manufacturer's instructions. In brief, a reaction mixture of 50 μ l contained 25 μ l of 2xSYBR Green PCR Master Mix (contains 5 mM MgCl₂, 200 μ M dATP, dCTP, dGTP, 400 μ M dUTP, 1.25 U AmpliTaq Gold DNA polymerase, 0.5 U AmpErase uracil N-glycosylase), 400 nM forward and reverse primers, and 4 μ l cDNA. The PCR reactions were set in a MicroAmp optical 96 well reaction plate with MicroAmp optical caps for 40 cycles (95°C for 15 seconds, 60°C for one minute) in the ABI Prism 7700 Sequence Detector (PE-Applied Biosystems, Foster City, CA), the fluorescent signals (ΔR_n) at the threshold cycle were recorded by the machine built-in software, and target gene copies were calculated against the regression of the standard curve. To standardise target gene level with respect to the variability in RNA and cDNA quality, housekeeping gene 18S or glyceraldehyde-3-phosphate dehydrogenase was amplified in the same condition as an internal control. For interleukin 1 β (IL1 β) and tumour necrosis factor α (TNF α) gene expression, sense and antisense primer pairs specific for murine cytokine IL1 β and TNF α were reconstituted at a concentration of 4 μ mol/l. A serial dilution of target gene standard was included in each run for quantification of cytokine gene expression. For TCR V β expression, paired primers for 18 V β gene families based on previous published sequences^{19,20} were used. Expression of the TCR β chain constant region in each individual sample was used as reference to quantify the V β expression.

Statistical analysis

The incidence of arthritis between groups of mice was determined with the χ^2 test. Clinical and histological severity of arthritis was analysed with the non-parametric Mann-Whitney U test. Anti-CII antibody titres and cytokine expression were analysed with Student's *t* test.

RESULTS

Clinical characterisation of arthritis in B6.IF γ KO mice

To compare arthritis in B6.IF γ KO mice with classical CIA, DBA/1 mice were included in all experiments as disease

Figure 1 Arthritis in B6.IFN γ KO mice immunised with bovine CII in CFA. (A) An arthritic hind paw of a B6.IFN γ KO mouse at day 3 of arthritis with a clinical score 1. There is substantial erythematous oedema from the ankle through the digits. Note the marked swollen tarsal and metatarsal joints. This is the typical sign of an arthritic paw seen in CIA in DBA/1 mice. (B) Normal appearance of a non-arthritic hind paw of a B6 mouse immunised with bovine CII in CFA.

controls. All DBA/1 mice (30/30) in the experiments developed arthritis with a mean (SD) onset of 32 (6.7) days after immunisation. In three experiments 24/35 (69%, range 60–80%) B6.IFN γ KO mice developed arthritis with a similar mean onset at day 28 (4.7) to that of DBA/1 mice. Similar to the arthritis seen in DBA/1 mice, disease in B6.IFN γ KO mice affected both fore and hind limbs and was primarily focused in metacarpal and metatarsal joints and extended into the ankle or wrist and digits. The arthritis was characterised by erythematous oedema appearing in one or two limbs (fig 1). This joint swelling increased the paw thickness by 20–120%.

The clinical disease course was followed up for 35 days after the initial appearance of arthritis. Figure 2 illustrates the evolution of arthritis in B6.IFN γ KO mice. B6.IFN γ KO mice developed a monophasic progressive arthritis and most of the arthritic joints progressed from erythematous oedema (clinical score 1) to distortion (clinical score 2) and some progressed to ankylosis (clinical score 3) of the affected joints. This disease pattern is similar to that of DBA/1 mice. Some of the arthritic limbs in B6.IFN γ KO mice remitted spontaneously 7–21 days after the first sign of arthritis, and the remission rate of arthritic limbs in B6.IFN γ KO mice was higher than that seen in DBA/1 mice (14% v 7%). At the end of the experiments (day 35 of arthritis) a comparison of DBA/1 and B6.IFN γ KO mice showed a lower clinical score ($p < 0.05$) and fewer limbs affected ($p < 0.05$) in the B6.IFN γ KO group (fig 2). This suggests that B6.IFN γ KO mice have a less aggressive disease than classical CIA in DBA/1 mice.

Over the course of the three experiments, 3/30 (10%; 3/10 of a single group) B6 mice showed signs of arthritis within the duration of 65 days after immunisation. This finding was unexpected, as this strain has previously proved resistant to disease during many previous studies. The only noted variation that might be attributed to this outcome was the use of high levels of *Mycobacterium tuberculosis* supplementation within the adjuvant preparation. However, none of the B6 mice (0/5) injected with CFA in acetic acid alone showed any sign of arthritis. The three arthritic B6 mice developed a type of mild non-progressive disease. Arthritis was limited to one

Figure 2 Natural clinical course of arthritis in B6.IFN γ KO mice. The average clinical score of arthritic mice reflects the clinical severity of an individual arthritic limb and the number of affected limbs. DBA/1 mice exhibited a typical monophasic progressive disease. Similarly, B6.IFN γ KO mice showed a progressive type of arthritis. Most of the affected joints progressed from initial swelling to distortion and some affected joints progressed to ankylosis. The lower clinical score at the late stage of disease in B6.IFN γ KO mice reflects the smaller number of limbs affected and less severity of the affected limbs than in DBA/1 mice (* $p < 0.05$). B6 mice had a mild and transient arthritis affecting only one limb in each arthritic mouse.

limb in each of the affected mice, and all the arthritic limbs completely remitted 21 days after the first sign of arthritis, and macroscopically returned to a normal appearance. This clinical pattern was clearly different from the arthritis in either the B6.IFN γ KO or DBA/1 mice. The incidence of arthritis in wild-type B6 mice was significantly lower ($p < 0.01$) than that seen in either B6.IFN γ KO or DBA/1 mice.

Histological features of arthritis in B6.IFN γ KO mice

Arthritis in B6.IFN γ KO mice exhibited typical histological features as previously described in DBA/1 mice—namely, synovitis, pannus formation, marginal erosion, cartilage destruction, and architectural changes of the joint. Synovitis began with infiltration by neutrophils and mononuclear cells, with later joint infiltrates comprised predominantly by mononuclear cells (fig 3A). Marginal erosion was seen as early as the day of clinical arthritis onset. Pannus formation was a common feature of disease in B6.IFN γ KO mice (fig 3B), and was seen in 80% of the sections. Morphological changes of cartilage began with a decreased number of chondrocytes, which could be seen as early as day 1 of clinical arthritis. The content of proteoglycan in the cartilage was evaluated by staining of sections with aldehyde fuchsin. Loss of proteoglycan could be seen early in the joint disease, and extensive destruction of the joint architecture, complete loss of cartilage, bone erosion, and remodelling in severe arthritis occurred (fig 3C). All histopathological features of arthritis in B6.IFN γ KO mice were similar to those seen in arthritic joints in DBA/1 mice, although there were variations in severity between individual mice and individual arthritic joints. A comparison at day 35 of arthritis indicated that the overall histological score in

Figure 3 Histopathology of arthritis in B6.IFN γ KO mice. (A) Section of a hind paw with arthritis at the day of onset showing inflamed synovium with massive infiltrate of mononuclear cells. There is decreased number of chondrocytes in the articular cartilage. (B) Section of a hind paw with arthritis at day 14 showing severe synovitis, pannus formation, and erosion and some visible destruction of articular cartilage. (C) Section of a hind paw with arthritis at day 35 showing massive synovitis, erosion of cartilage and subchondral bone, and destruction of joint structure. (D) Normal appearance of a section of a hind paw from a non-arthritic CII immunised B6 mouse. (E) Section of a remitted arthritic hind paw from a B6 mouse showing normal joint appearance. (F) Histological assessment of arthritis in affected limbs in B6.IFN γ KO mice (open bars) at the end of the experiments (day 35 of arthritis) in comparison with that in DBA/1 mice (filled bars). S, synovitis; P, pannus formation; E, erosion; A, architectural changes; O, overall histological score. * $p < 0.01$; ** $p < 0.05$.

B6.IFN γ KO mice was lower than in DBA/1 mice (fig 3F, $p < 0.05$). This suggests that B6.IFN γ KO mice developed a slightly less severe arthritis than that in DBA/1 mice, which is in agreement with the clinical findings.

Levels of IgG1 and IgG2b anti-murine CII antibodies in B6.IFN γ KO mice

No significant differences in serum levels of total IgG anti-bovine CII and murine CII antibodies were seen between the three strains of mice. Because IFN γ mediates antibody class switch to IgG2a, B6.IFN γ KO mice produced only minimal amount of IgG2a to either bovine or murine CII (fig 4) after bovine CII immunisation. However, production of IgG2b and IgG1 autoantibodies to murine CII was significantly increased in B6.IFN γ KO mice as compared with those in B6 mice. B6 mice generated higher levels of IgG2a autoantibodies to murine CII than B6.IFN γ KO mice, but the levels were significantly lower than the levels in DBA/1 mice (fig 4). A slight

increase in the levels of IgM antibodies to both bovine and murine CII was detected in B6.IFN γ KO mice at 14 day after immunisation as compared with DBA/1 and B6 mice; however, these differences were not statistically significant. The levels of anti-CII IgG3, IgA and IgE antibodies in the three groups of mice were also measured and no significant difference was seen in any of the classes between the three groups of mice (not shown).

Enhanced cellular proliferation in B6.IFN γ KO mice

Draining lymph node cells from immunised mice were isolated and cultured in T cell medium and proliferation in response to concanavalin A and CII was evaluated. Lymph node cells from B6.IFN γ KO mice showed increased baseline proliferation in medium with no stimuli added. This was consistent with previous findings that T cells from antigen and CFA immunised B6.IFN γ KO mice proliferate spontaneously during *in vitro* culture.²¹ After concanavalin A stimulation,

Figure 4 Levels of anti-heterologous and autologous CII antibodies in B6.IFN γ KO mice immunised with bovine CII in CFA (n=10 mice in each group). *p<0.01 (B6.IFN γ KO v B6); **p<0.05 (B6.IFN γ KO v B6).

lymphocytes from B6.IFN γ KO mice showed substantially increased proliferation compared with cells from DBA/1 and B6 mice (p<0.01). When restimulated with bovine CII, B6.IFN γ KO lymphocyte proliferation increased by 20–30% above the baseline level in a dose dependent manner (fig 5).

Levels of IL1 β and TNF α gene expression in arthritic joints in B6.IFN γ KO and DBA/1 mice

Using quantitative real-time RT-PCR, we determined gene expression of both IL1 β and TNF α in arthritic joints. DBA/1 mice exhibited high expression of both IL1 β and TNF α within arthritic joints, and comparable levels of both inflammatory cytokine genes were detected in arthritic joints from B6.IFN γ KO mice (fig 6). However, only negligible expression of both cytokines was found in the joints with remitted arthritis from B6 mice, and clinical non-arthritic joints of both DBA/1 and B6.IFN γ KO mice.

Figure 5 Enhanced proliferation of lymphocytes from CII immunised B6.IFN γ KO mice. Results are representative of three experiments; n=3 in each group.

Expression of TCR V β 6 and V β 8 in B6.IFN γ KO arthritic joints

A quantitative real-time RT-PCR technique was used to analyse TCR V β expression in arthritic joints. Strong C β signals were obtained using preliminary RT-PCR from RNA preparation of arthritic paws and no signal of C β was obtained from those with no clinical signs of arthritis. These findings were in agreement with previous analyses of murine arthritis²⁰; therefore cDNA from unaffected paws was not analysed further by real-time RT-PCR.

Figure 6 Expression of cytokines in arthritic joints in B6.IFN γ KO mice. IL1 β and TNF α gene expression was quantified using a quantitative real-time RT-PCR. Levels of IL1 β and TNF α gene expression in arthritic joints in B6.IFN γ KO are comparable with those in DBA/1 mice (p>0.05). Affected and remitted joints in B6 mice had negligible levels of expression of IL1 β and TNF α (p<0.01) compared with those in DBA/1 or B6.IFN γ KO mice.

Figure 7 Predominant TCR V β 6 and V β 8 usage in arthritic joints in B6.IFN γ KO mice. Expression of TCR V β gene expression was determined by quantitative real-time RT-PCR. This composite graph shows individual arthritic mouse samples from both DBA/1 (top, n=9) and B6.IFN γ KO (bottom, n=12) mice. The V β gene expression was expressed as a relative quantity in reference to the C β expression in the same sample. In DBA/1 mice, V β 6 had a predominant expression over the other V β genes in most of the samples. In B6.IFN γ KO mice, V β 6 had a two- to 50-fold increased expression over other V β genes in 10 of 12 samples. In addition, the V β 8 gene family exhibited the second most increased expression in most V β 6 predominant samples. In the two samples in which V β 6 was not predominant, V β 8.1 and V β 8.2 outnumbered V β 6 as the predominantly expressed V β genes.

We used a library of 21 pairs of TCR V β primers to phenotype the T cells in arthritic joints. All the 21 TCR V β subsets were present at high levels in draining lymph nodes from mice of three groups immunised with CII in CFA using this technique, with the exception of V β 17, which is genetically deleted in DBA/1 mice. Figure 7 shows an analysis of the TCR V β expression in arthritic paws from DBA/1 and B6.IFN γ KO mice. Results were expressed as the relative quantity (copy number) of V β gene expression as compared with C β expression within the same cDNA preparation. Thirteen out of 21 V β were detected in DBA/1 arthritic paws: V β 2, 3, 4, 6, 8.1, 8.2, 8.3, 9, 10, 11, 14, 15, and 16. V β 6 was readily detected in all arthritic joints, and moreover, the quantity of V β 6 expression grossly outweighed all other V β phenotypes. This finding was consistent in all but one arthritic joint of DBA/1 arthritic mice.

Nineteen of the 21 V β types were detected in B6.IFN γ KO mice, with V β 17 and V β 18 being absent. Similar to the observation in DBA/1 arthritic paws, V β 6 was readily detected in all cDNA samples from B6.IFN γ KO mice. In addition, V β 8 and V β 6 were more frequently detected than other V β genes in B6.IFN γ KO mice. The level of V β 6 expression was much higher than that of any other V β genes in 10 of 12 arthritic samples. V β 6 had a two- to 50-fold increased expression over

other V β genes in the same arthritic joint. In one sample, only V β 6 and V β 8.2 were detected, and the V β 6 level was 48-fold higher than that of V β 8.2. In the two samples without V β 6 predominance, one had very high V β 8.2 expression and the other had high levels of V β 8.1. Overall, the V β 8 family was detected at a frequency only exceeded by V β 6, and occurred in 11 of the 12 samples.

DISCUSSION

These findings demonstrate that arthritis develops in B6 mice lacking IFN γ production owing to transgenic manipulations, and the disease that occurs is an inflammatory, erosive arthritis similar to classical CIA in DBA/1 mice. Examination of the natural clinical course of the disease and subsequent histological analysis disclosed a slightly less aggressive disease in B6.IFN γ KO mice than in DBA/1 mice. CIA in B6.IFN γ KO mice is also characterised by strong humoral and cellular immune response to autologous CII, and the production of high levels of proinflammatory cytokines in arthritic joints. Most interestingly, B6.IFN γ KO mice have a similar restricted TCR V β profile in arthritic joints to the pattern seen in DBA/1 mice.

Many factors are known to influence the development of CIA in mice.²² Most notable is that susceptibility to CIA is

strongly associated with the expression of specific MHC, leading to the observation that mice bearing H-2^a or H-2^r are susceptible to disease, whereas those bearing other H-2 haplotypes (such as H-2^b) are resistant. It is now apparent that this key level of genetic regulation may be circumvented, and that cytokines represent a means to escape immunogenetic regulation. IFN γ is a Th1 cytokine that has been generally considered to have a role in promoting disease in CIA. However, as shown in the present report and by others, genetic disruption of IFN γ signalling converts the resistant strains of mice (H-2^b and H-2^d) to be highly susceptible to CIA induction.^{16, 23} This clearly suggests that IFN γ has a role in suppressing disease in CIA. The profound effect on susceptibility to CIA by disruption of the IFN γ pathway has been partially attributed to increased expression of IL12 and IL1 β . However, IL12 and IL1 β are proinflammatory cytokines shown to participate in promoting inflammation in CIA in mice with an intact IFN γ pathway. It is therefore not surprising that blocking either IL12 or IL1 β can reduce the incidence and severity of arthritis in B6.IFN γ KO mice. As an alternative hypothesis, Matthys *et al* suggested that enhanced CIA in IFN γ R KO DBA/1 mice might be related to CFA induced expansion of Mac1⁺ myeloid cells.²⁴

We have previously shown that IFN γ is involved in the elimination of activated CD4⁺ T cells.^{21, 25} Failure to suppress the expansion of activated CD4⁺ T cells in B6.IFN γ KO mice leads to a more severe and chronic progressive disease in experimental allergic encephalomyelitis.²¹ IFN γ suppresses activated CD4⁺ T cell activity by inhibiting proliferation and promoting apoptosis, illustrated by both in vitro findings and in vivo observations at the site of inflammation in IFN γ KO mice.²¹ In DBA/1 IFN γ R KO mice primed with CII in CFA, delayed-type hypersensitivity reactions to CII were enhanced,²⁴ which further supports an immunosuppressive activity of IFN γ in vivo. Wild-type B6 mice can mount strong T cell responses to CII and produce IgG2a subclass autoantibodies to murine CII, but they fail to develop clinical arthritis. One possible explanation is that the infiltration or proliferation of activated CD4⁺ T cells within joints of B6 mice is inhibited by IFN γ , and subsequently the cells are eliminated from the joint or systemic circulation through IFN γ mediated apoptosis. It would be of interest to investigate whether there is a defect in the IFN γ pathway in CIA susceptible strains of mice such as DBA/1 and B10.Q mice.

The other explanation of arthritis susceptibility in these IFN γ KO mice is a dysregulation of chemokines in B6.IFN γ KO, resulting in CII-specific T cell migration to, and retention in, the joint tissue. Matthys *et al* reported that CXCR4 is highly expressed by leucocytes isolated from arthritic joints of CIA in DBA/1 IFN γ R KO mice.²⁶ Blocking interaction of CXCR4 with its ligand (stromal cell derived factor 1) reduced the severity of CIA and the delayed-type hypersensitivity response to CII in mice. However, the expression of CXCR4 by CD4⁺ T cells using TCR V β 6 or V β 8 in lymph nodes and in arthritic joints is currently unknown.

The production of autoantibodies to murine CII is a critical immunological feature in CIA. These autoantibodies, in particular complement fixing IgG2 antibodies, seem to be important in the initiation of disease. Despite a minimal amount of IgG2a anti-murine CII antibodies, B6.IFN γ KO mice could still develop extensive chronic arthritis. This may be attributed to high levels of IgG2b production in these mice, because IgG2b anti-murine CII antibodies can initiate arthritis in B10.Q mice.^{27, 28} IFN γ KO mice generate high titres of IgG1 antibodies after antigen challenge or infection (Chu and Dalton, unpublished observations).²⁹ In agreement with this observation, we found that B6.IFN γ KO mice produced significantly increased levels of IgG1 anti-murine CII autoantibodies. However, the significance of these autoantibodies in the development of CIA is unclear at present.

TCR V β genes exert a dominant influence upon CIA susceptibility in mice. Using TCR V β congenic B10.Q (H-2^a) mice,

Nabozny *et al* identified T cells using V β 6 that contribute to arthritis in CIA.⁵ A restricted TCR V β usage in arthritic joints in CIA has been demonstrated for both V β 6 and V β 8 in mice of H-2^a and H-2^r haplotypes.^{2, 4, 19} In this study we demonstrate the predominant expression of V β 6 in arthritic DBA/1 joints. Surprisingly, we found that B6.IFN γ KO (H-2^b) mice also showed a similar predominant expression of V β 6 in arthritic joints. In addition, the V β 8 family was the second most abundant V β phenotype expressed in arthritic joints from B6.IFN γ KO mice. In contrast, the TCR V β expression in draining lymph nodes from immunised mice showed no such restricted pattern, and there was no difference between TCR V β expression patterns in draining lymph nodes from CII immunised B6.IFN γ KO and wild-type B6 mice. This indicates that "arthritogenic" T cells exist in CIA resistant mouse strains, and may suggest that preferential recruitment of arthritogenic T cells to the joint in the absence of IFN γ in B6 mice can occur.

Previous studies have identified the restricted T cell epitopes of the CII molecule in association with H-2^a.^{8, 30} These epitopes stimulate proliferation of T cells from CIA susceptible congenic strains such as DBA/1 mice, but not those from non-susceptible strains. This indicates that these epitopes have an important role in disease initiation. Interestingly, T cells from another CIA susceptible strain of mice of the H-2^r haplotype respond to epitopes that are different from those in H-2^a mice.³¹ IFN γ is recognised to up regulate MHC molecule expression on antigen-presenting cells,³² but it is not known whether IFN γ influences TCR-epitope-H-2 interaction. It will be important to compare T cell epitopes that are recognised by B6.IFN γ KO mice with those well characterised epitopes recognised by H-2^a and H-2^r mice.

Overall, susceptibility to CIA in B6.IFN γ KO mice demonstrates that MHC regulation of this experimental disease is not an absolute phenomenon. The similarity of the pathological and immunological features of the disease to those seen in classical CIA suggests that this is not a joint disease arising from genetic manipulation, as previously reported for other models.^{33, 34} Further, our findings indicate that autoimmune T cell subsets previously implicated in experimental arthritis are expressed in this H-2^b transgenic mouse strain, and infiltrate the joint in a similar manner to T cells in H-2^a DBA/1 mice.

ACKNOWLEDGEMENTS

Dr. Cong-Qiu Chu was supported by the NIH Physician-Immunologist Training Programme (grant number AI07527-05), Wayne State University School of Medicine.

Authors' affiliations

C-Q Chu, Department of Internal Medicine, Wayne State University School of Medicine, Detroit, MI 48201, USA

Z Song, L Mayton, B Wu, P H Wooley, Department of Orthopaedic Surgery, Wayne State University School of Medicine, Detroit, MI 48201, USA

REFERENCES

- 1 **Wooley PH**, Luthra HS, Stuart JM, David CS. Type II collagen-induced arthritis in mice. I. Major histocompatibility complex (I region) linkage and antibody correlates. *J Exp Med* 1981;154:688-700.
- 2 **Andersson M**, Jansson L, Holmdahl R. Role for T-cell receptor V β genes in collagen induced arthritis. *Immunogenetics* 1992;35:71-2.
- 3 **Banerjee S**, Haqqi TM, Luthra HS, Stuart JM, David CS. Possible role of V beta T cell receptor genes in susceptibility to collagen-induced arthritis in mice. *J Exp Med* 1988;167:832-9.
- 4 **Griffiths MM**, Cole BC, Ito J, Harper DS, Anderson GD, Cannon GW, *et al*. T-cell receptors and collagen induced arthritis in H-2r mice. *Autoimmunity* 1993;14:221-9.
- 5 **Nabozny GH**, Bull MJ, Hanson J, Griffiths MM, Luthra HS, David CS. Collagen-induced arthritis in T cell receptor V beta congenic B10.Q mice. *J Exp Med* 1994;180:517-24.
- 6 **Ranges GE**, Sriram S, Cooper SM. Prevention of type II collagen-induced arthritis by in vivo treatment with anti-L3T4. *J Exp Med* 1985;162:1105-10.
- 7 **David CS**. Genes for MHC, TCR and MIs determine susceptibility to collagen induced arthritis. *APMIS* 1990;98:575-84.

- 8 **Myers LK**, Seyer JM, Stuart IM, Terato K, David CS, Kang AH. T cell epitopes of type II collagen that regulate murine collagen-induced arthritis. *J Immunol* 1993;151:500–5.
- 9 **Holmdahl R**, Jansson L, Gullberg D, Rubin K, Forsberg PO, Klareskog L. Incidence of arthritis and autoreactivity of anti-collagen antibodies after immunization of DBA/1 mice with heterologous and autologous collagen II. *Clin Exp Immunol* 1985;62:639–46.
- 10 **Cooper SM**, Sircam S, Ranges GE. Suppression of murine collagen-induced arthritis with monoclonal anti-Ia antibodies and augmentation with IFN-gamma. *J Immunol* 1988;141:1958–62.
- 11 **Boissier MC**, Chiochia G, Bessis N, Hajnal J, Garotta G, Nicoletti F, et al. Biphasic effect of interferon-gamma in murine collagen-induced arthritis. *Eur J Immunol* 1995;25:1184–90.
- 12 **Williams RO**, Williams DG, Feldmann M, Maini RN. Increased limb involvement in murine collagen-induced arthritis following treatment with anti-interferon-gamma. *Clin Exp Immunol* 1993;92:323–7.
- 13 **Nakajima H**, Hiyama Y, Takamori H, Tsukada W. Cell-mediated transfer of collagen-induced arthritis in mice and its application to the analysis of the inhibitory effects of interferon-gamma and cyclophosphamide. *Clin Exp Immunol* 1993;92:328–35.
- 14 **Manoury-Schwartz B**, Chiochia G, Bessis N, Abelsira-Amar O, Batteux F, Muller S, et al. High susceptibility to collagen-induced arthritis in mice lacking IFN-gamma receptors. *J Immunol* 1997;158:5501–6.
- 15 **Vermeire K**, Heremans H, Vandeputte M, Huang S, Billiau A, Matthys P. Accelerated collagen-induced arthritis in IFN-gamma receptor-deficient mice. *J Immunol* 1997;158:5507–13.
- 16 **Ortmann RA**, Shevach EM. Susceptibility to collagen-induced arthritis: cytokine-mediated regulation. *Clin Immunol* 2001;98:109–18.
- 17 **Wooley PH**, Cingel B. Staphylococcal enterotoxin B increases the severity of type II collagen induced arthritis in mice. *Ann Rheum Dis* 1995;54:298–304.
- 18 **Mosman T**. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;65:56–63.
- 19 **Haqqi TM**, Anderson GD, Banerjee S, David CS. Restricted heterogeneity in T-cell antigen receptor V beta gene usage in the lymph nodes and arthritic joints of mice. *Proc Natl Acad Sci USA* 1992;89:1253–5.
- 20 **Wooley PH**, Sud S, Whalen JD, Nasser S. Pristane-induced arthritis in mice. V. Susceptibility to pristane-induced arthritis is determined by the genetic regulation of the T cell repertoire. *Arthritis Rheum* 1998;41:2022–31.
- 21 **Chu CQ**, Wittmer S, Dalton DK. Failure to suppress the expansion of the activated CD4 T cell population in interferon gamma-deficient mice leads to exacerbation of experimental autoimmune encephalomyelitis. *J Exp Med* 2000;192:123–8.
- 22 **Staines NA**, Wooley PH. Collagen arthritis—what can it teach us? *Br J Rheumatol* 1994;33:798–807.
- 23 **Guedez YB**, Whittington KB, Clayton JL, Joosten LA, van de Loo FA, van den Berg WB, et al. Genetic ablation of interferon-gamma up-regulates interleukin-1beta expression and enables the elicitation of collagen-induced arthritis in a nonsusceptible mouse strain. *Arthritis Rheum* 2001;44:2413–24.
- 24 **Matthys P**, Vermeire K, Mitera T, Heremans H, Huang S, Schols D, et al. Enhanced autoimmune arthritis in IFN-gamma receptor-deficient mice is conditioned by mycobacteria in Freund's adjuvant and by increased expansion of Mac-1+ myeloid cells. *J Immunol* 1999;163:3503–10.
- 25 **Dalton DK**, Haynes L, Chu CQ, Swain SL, Wittmer S. Interferon gamma eliminates responding CD4 T cells during mycobacterial infection by inducing apoptosis of activated CD4 T cells. *J Exp Med* 2000;192:117–22.
- 26 **Matthys P**, Hatse S, Vermeire K, Wuyts A, Bridger G, Henson GW, et al. Amd3100, a potent and specific antagonist of the stromal cell-derived factor-1 chemokine receptor CXCR4, inhibits autoimmune joint inflammation in IFN-gamma receptor-deficient mice. *J Immunol* 2001;167:4686–92.
- 27 **Watson WC**, Townes AS. Genetic susceptibility to murine collagen II autoimmune arthritis. Proposed relationship to the IgG2 autoantibody subclass response, complement C5, major histocompatibility complex (MHC) and non-MHC loci. *J Exp Med* 1985;162:1878–91.
- 28 **Wooley PH**, Luthra HS, Krco CJ, Stuart JM, David CS. Type II collagen-induced arthritis in mice. II. Passive transfer and suppression by intravenous injection of anti-type II collagen antibody or free native type II collagen. *Arthritis Rheum* 1984;27:1010–17.
- 29 **Graham MB**, Dalton DK, Giltinan D, Braciale VL, Stewart TA, Braciale TJ. Response to influenza infection in mice with a targeted disruption in the interferon gamma gene. *J Exp Med* 1993;178:1725–32.
- 30 **Myers LK**, Terato K, Seyer JM, Stuart JM, Kang AH. Characterization of a tolerogenic T cell epitope of type II collagen and its relevance to collagen-induced arthritis. *J Immunol* 1992;149:1439–43.
- 31 **Myers LK**, Miyahara H, Terato K, Seyer JM, Stuart JM, Kang AH. Collagen-induced arthritis in B10.RIII mice (H-2r): identification of an arthritogenic T-cell determinant. *Immunology* 1995;84:509–13.
- 32 **Boehm U**, Klamp T, Groot M, Howard JC. Cellular responses to interferon-gamma. *Annu Rev Immunol* 1997;15:749–95.
- 33 **Butler DM**, Malfait AM, Mason LJ, Warden PJ, Kollias G, Maini RN, et al. DBA/1 mice expressing the human TNF-alpha transgene develop a severe, erosive arthritis: characterization of the cytokine cascade and cellular composition. *J Immunol* 1997;159:2867–76.
- 34 **Saggioro D**, Rosato A, Esposito G, Rosenberg MP, Harrison J, Felber BK, et al. Inflammatory polyarthropathy and bone remodeling in HTLV-I Tax- transgenic mice. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997;14:272–80.