EXTENDED REPORT

IFNγ deficient C57BL/6 (H-2^b) mice develop collagen induced arthritis with predominant usage of T cell receptor Vβ6 and Vβ8 in arthritic joints

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Background: Transgenic deficiency in interferon γ (IFNγ) or IFNγ 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) Vβ 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 IFNγ knockout C57BL/6 (B6.IFNγ KO) mice (H-2^b) were compared directly with those in DBA/1 mice (H-2^q).
Results: 60–80% of B6.IFNγ KO mice developed a progressive arthritis with a similar clinical course to classical CIA in DBA/1 mice. The affected joints in B6.IFNγ KO mice had an erosive form of arthritis with similar features to joint disease in DBA/1 mice. B6.IFNγ 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 1β and tumour necrosis factor α expression were detected in arthritic joints from B6.IFNγ KO and DBA/1 mice. B6.IFNγKO mice used predominantly TCR Vβ6 and Vβ8 in arthritic joints. This TCR Vβ profile is similar to that found in DBA/1 mice with CIA.
Conclusions: C57BL/6 mice deficient in IFNγ production can develop arthritis that resembles classical CIA. These data suggest that IFNγ 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^q or H-2^r 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^q or H-2^r class II MHC molecules to "autoimmune" T cell subsets expressing predominantly Vβ6 and Vβ8.1 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 (IFNγ) in the development of CIA has been a focus of several studies. IFNγ has been used as a signature cytokine for Th1 cells because it is produced mainly by activated CD4$^+$ T cells and NK cells. Theoretically, IFNγ is believed to be pathogenic in CIA and there is evidence supporting this notion. Cooper *et al* showed that IFNγ 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 IFNγ increased disease severity in arthritic mice.[11] However, other studies have suggested a protective role of IFNγ in CIA. Treatment of CIA with anti-IFNγ antibodies increased limb involvement in mice, although anti-IFNγ 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 IFNγ also inhibited adoptive arthritis upon transfer with CII immunised spleen cells.[13] Recent studies using IFNγ receptor knockout (KO) mice on the CIA susceptible DBA/1 background suggested a beneficial role of IFNγ in this experimental disease. IFNγ 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 IFNγ gene might break the resistance to CIA induction in strains of mice bearing other H-2 haplotypes than H-2^q or H-2^r. Ortmann and Shevach recently reported that arthritis could be induced in IFNγ deficient C57BL/6 (H-2^b), IFNγ deficient BALB/c (H-2^d), or IFNγ 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 IFNγ KO C57BL/6 (B6.IFNγ 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 Vβ 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; IFNγ, interferon γ; IL1β, interleukin 1β; 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; TNFα, tumour necrosis factor α

MATERIALS AND METHODS

Mice

Female DBA/1, B6.129S7-ifng^{tm1Ts} (B6.IFNγ 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 μg 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 previously[1]: 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 μg/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-mercapitol ethanol, 2 mM L-glutamine) in the presence of native bovine CII (10 and 100 μg/ml) diluted in medium, concanavalin A (3 μg/ml), or medium alone. The cells were plated at 4×10^5/well in triplicate in 96 well plates for 24 hours, and 10 μl of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) solution (20 μg/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 μl 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 μg total RNA in a 40 μl reaction mixture containing 500 μmol/l each of deoxynucleotide triphosphates, 0.4 U/μl RNAase inhibitor, 2.5 μM 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 μl contained 25 μl of 2xSYBR Green PCR Master Mix (contains 5 mM $MgCl_2$, 200 μM dATP, dCTP, dGTP, 400 μM dUTP, 1.25 U AmpliTaq Gold DNA polymerase, 0.5 U AmpErase uracil *N*-glycosylase), 400 nM forward and reverse primers, and 4 μl 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 (ΔRn) 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 1β (IL1β) and tumour necrosis factor α (TNFα) gene expression, sense and antisense primer pairs specific for murine cytokine IL1β and TNFα were reconstituted at a concentration of 4 μmol/l. A serial dilution of target gene standard was included in each run for quantification of cytokine gene expression. For TCR Vβ expression, paired primers for 18 Vβ 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 Vβ 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.IFNγ KO mice

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

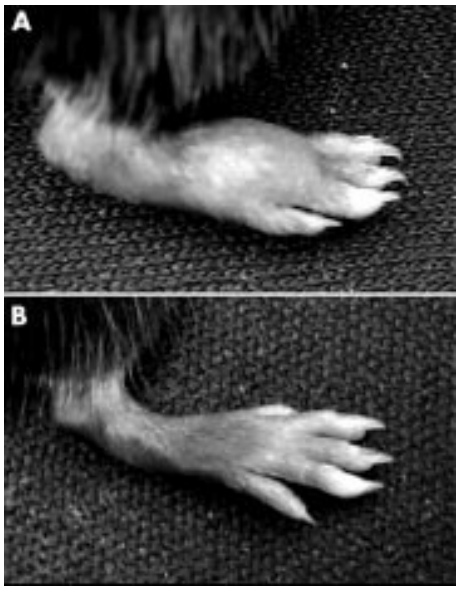

Figure 1 Arthritis in B6.IFNγ KO mice immunised with bovine CII in CFA. (A) An arthritic hind paw of a B6.IFNγ KO mouse at day 3 of arthritis with a clinical score 1. There is substantial erythematous oedema from the ankle through the digits. Note the marked swollen tarsal and metatarsal joints. This 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.

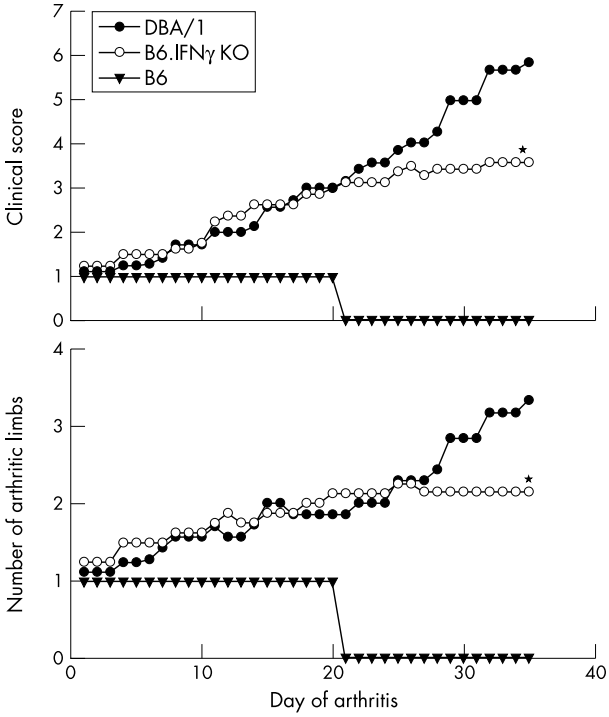

Figure 2 Natural clinical course of arthritis in B6.IFNγ KO mice. The average clinical score of arthritic mice reflects the clinical severity of an individual arthritic limb and the number of affected limbs. DBA/1 mice exhibited a typical monophasic progressive disease. Similarly, B6.IFNγ KO mice showed 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.IFNγ 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.

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.IFNγ 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.IFNγ KO mice affected both fore and hind limbs and was primarily focused in metarcarpal and metartarsal 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.IFNγ KO mice. B6.IFNγ 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.IFNγ KO mice remitted spontaneously 7–21 days after the first sign of arthritis, and the remission rate of arthritic limbs in B6.IFNγ 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.IFNγ KO mice showed a lower clinical score ($p<0.05$) and fewer limbs affected ($p<0.05$) in the B6.IFNγ KO group (fig 2). This suggests that B6.IFNγ 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 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.IFNγ 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.IFNγ KO or DBA/1 mice.

Histological features of arthritis in B6.IFNγ KO mice

Arthritis in B6.IFNγ 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.IFNγ 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 fuscia. 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.IFNγ 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

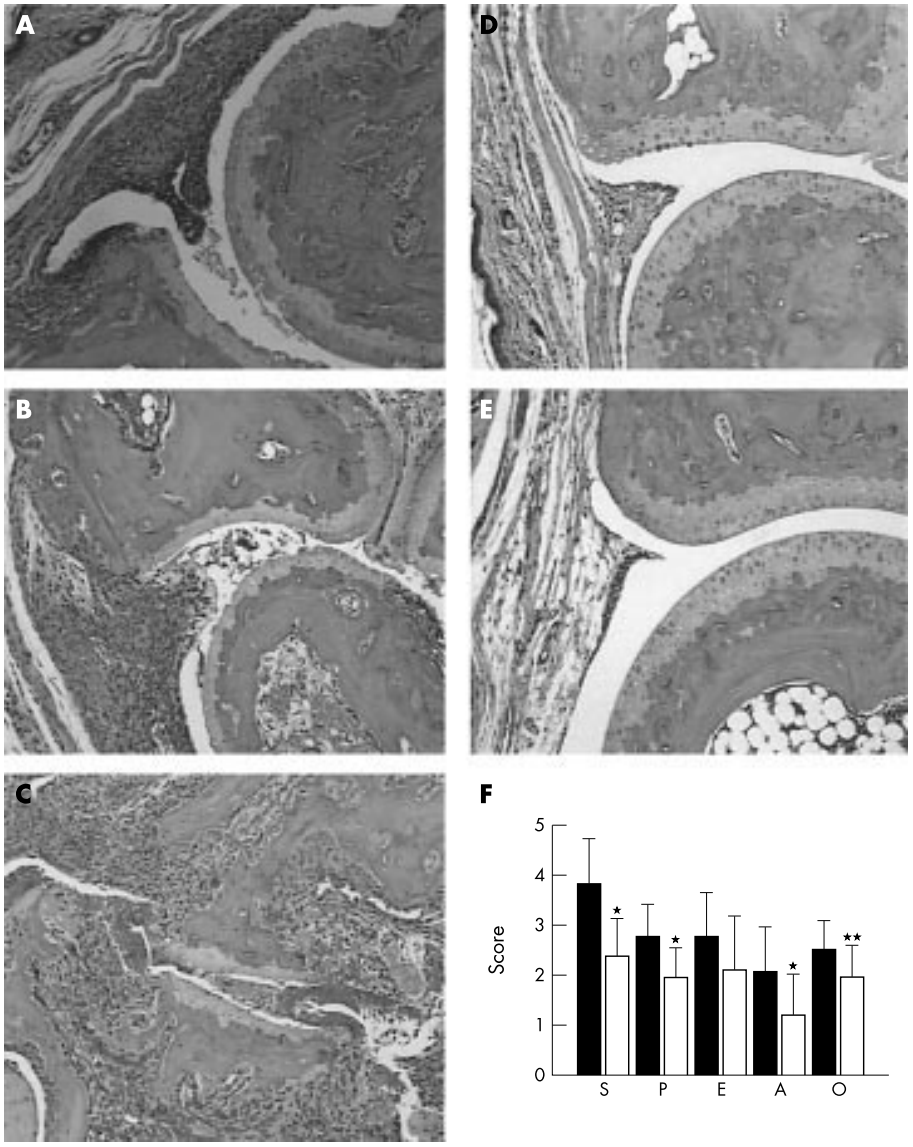

Figure 3 Histopathology of arthritis in B6.IFNγ KO mice. (A) Section of a hind paw with arthritis at the day of onset showing inflamed synovium with massive infiltrate of mononuclear cells. There is decreased number of chondrocytes in the articular cartilage. (B) Section of a hind paw with arthritis 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.IFNγ 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.IFNγ KO mice was lower than in DBA/1 mice (fig 3F, $p<0.05$). This suggests that B6.IFNγ 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.IFNγ KO mice

No significant differences in serum levels of total IgG anti-bovine CII and murine CII antibodies were seen between the three stains of mice. Because IFNγ mediates antibody class switch to IgG2a, B6.IFNγ 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.IFNγ KO mice as compared with those in B6 mice. B6 mice generated higher levels of IgG2a autoantibodies to murine CII than B6.IFNγ 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.IFNγ 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.IFNγ 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.IFNγ 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.IFNγ KO mice proliferate spontaneously during in vitro culture.[21] After concanavalin A stimulation,

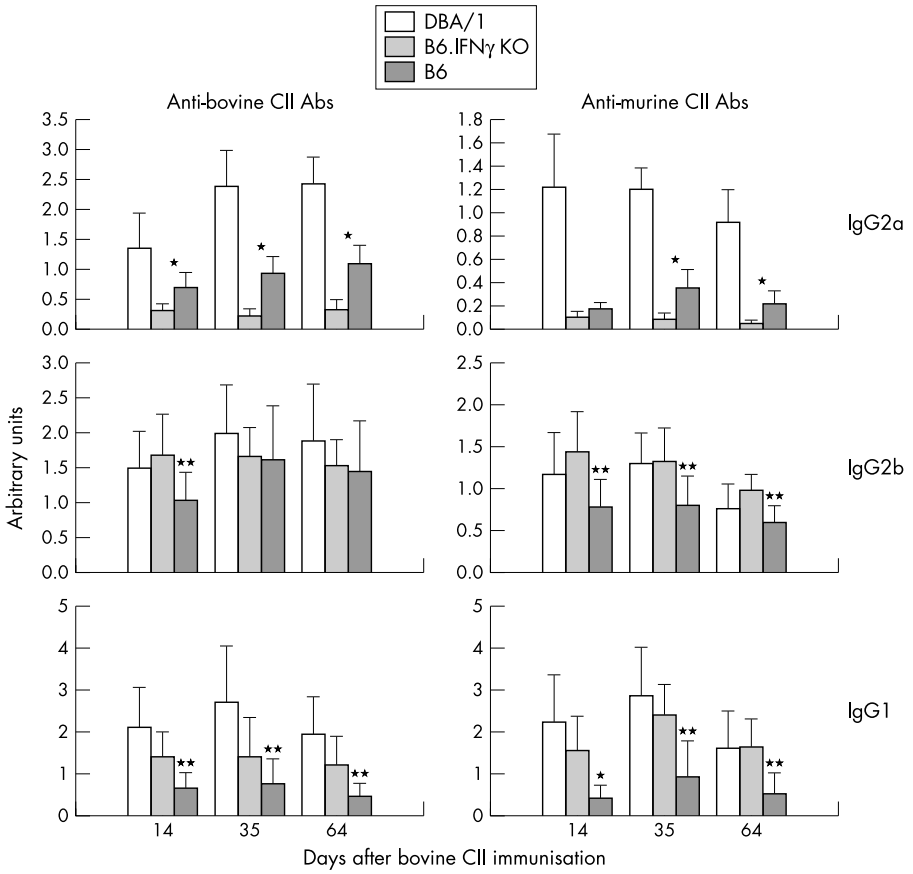

Figure 4 Levels of anti-heterologous and autologous CII antibodies in B6.IFNγ KO mice immunised with bovine CII in CFA (n=10 mice in each group). *p<0.01 (B6.IFNγ KO *v* B6); **p<0.05 (B6.IFNγ KO *v* B6).

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

Levels of IL1β and TNFα gene expression in arthritic joints in B6.IFNγ KO and DBA/1 mice

Using quantitative real-time RT-PCR, we determined gene expression of both IL1β and TNFα in arthritic joints. DBA/1 mice exhibited high expression of both IL1β and TNFα within arthritic joints, and comparable levels of both inflammatory cytokine genes were detected in arthritic joints from B6.IFNγ 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.IFNγ KO mice.

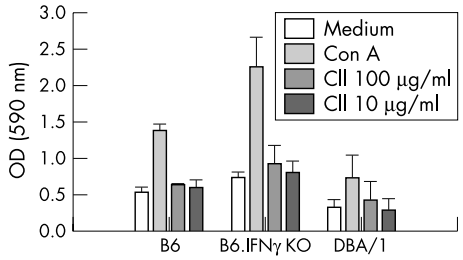

Figure 5 Enhanced proliferation of lymphocytes from CII immunised B6.IFNγ KO mice. Results are representative of three experiments; n=3 in each group.

Expression of TCR Vβ6 and Vβ8 in B6.IFNγ KO arthritic joints

A quantitative real-time RT-PCR technique was used to analyse TCR Vβ expression in arthritic joints. Strong Cβ signals were obtained using preliminary RT-PCR from RNA preparation of arthritic paws and no signal of Cβ was obtained from those with no clinical signs of arthritis. These findings were in agreement with previous analyses of murine arthritis[20]; therefore cDNA from unaffected paws was not analysed further by real-time RT-PCR.

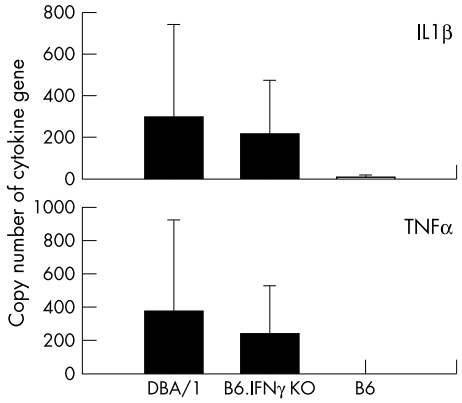

Figure 6 Expression of cytokines in arthritic joints in B6.IFNγ KO mice. IL1β and TNFα gene expression was quantified using a quantitative real-time RT-PCR. Levels of IL1β and TNFα gene expression in arthritic joints in B6.IFNγ KO are comparable with those in DBA/1 mice ($p>0.05$). Affected and remitted joints in B6 mice had negligible levels of expression of IL1β and TNFα ($p<0.01$) compared with those in DBA/1 or B6.IFNγ KO mice.

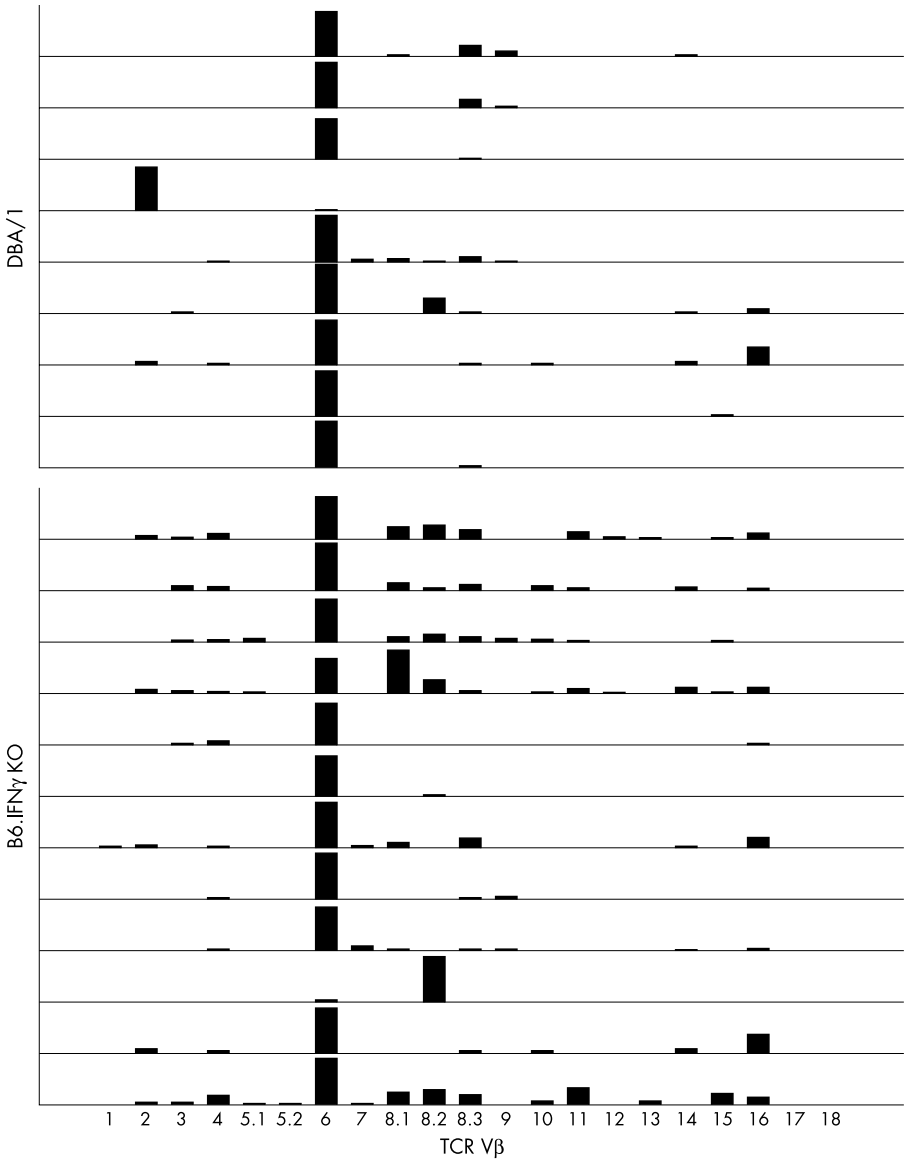

Figure 7 Predominant TCR Vβ6 and Vβ8 usage in arthritic joints in B6.IFNγ KO mice. Expression of TCR Vβ gene expression was determined by quantitative real-time RT-PCR. This composite graph shows individual arthritic mouse samples from both DBA/1 (top, n=9) and B6.IFNγ KO (bottom, n=12) mice. The Vβ gene expression was expressed as a relative quantity in reference to the Cβ expression in the same sample. In DBA/1 mice, Vβ6 had a predominant expression over the other Vβ genes in most of the samples. In B6.IFNγ KO mice, Vβ6 had a two- to 50-fold increased expression over other Vβ genes in 10 of 12 samples. In addition, the Vβ8 gene family exhibited the second most increased expression in most Vβ6 predominant samples. In the two samples in which Vβ6 was not predominant, Vβ8.1 and Vβ8.2 outnumbered Vβ6 as the predominantly expressed Vβ genes.

We used a library of 21 pairs of TCR Vβ primers to phenotype the T cells in arthritic joints. All the 21 TCR Vβ 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 Vβ17, which is genetically deleted in DBA/1 mice. Figure 7 shows an analysis of the TCR Vβ expression in arthritic paws from DBA/1 and B6.IFNγ KO mice. Results were expressed as the relative quantity (copy number) of Vβ gene expression as compared with Cβ expression within the same cDNA preparation. Thirteen out of 21 Vβ were detected in DBA/1 arthritic paws: Vβ2, 3, 4, 6, 8.1, 8.2, 8.3, 9, 10, 11, 14, 15, and 16. Vβ6 was readily detected in all arthritic joints, and moreover, the quantity of Vβ6 expression grossly outweighed all other Vβ phenotypes. This finding was consistent in all but one arthritic joint of DBA/1 arthritic mice.

Nineteen of the 21 Vβ types were detected in B6.IFNγ KO mice, with Vβ17 and Vβ18 being absent. Similar to the observation in DBA/1 arthritic paws, Vβ6 was readily detected in all cDNA samples from B6.IFNγ KO mice. In addition, Vβ8 and Vβ6 were more frequently detected than other Vβ genes in B6.IFNγ KO mice. The level of Vβ6 expression was much higher than that of any other Vβ genes in 10 of 12 arthritic samples. Vβ6 had a two- to 50-fold increased expression over other Vβ genes in the same arthritic joint. In one sample, only Vβ6 and Vβ8.2 were detected, and the Vβ6 level was 48-fold higher than that of Vβ8.2. In the two samples without Vβ6 predominance, one had very high Vβ8.2 expression and the other had high levels of Vβ8.1. Overall, the Vβ8 family was detected at a frequency only exceeded by Vβ6, and occurred in 11 of the 12 samples.

DISCUSSION

These findings demonstrate that arthritis develops in B6 mice lacking IFNγ 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.IFNγ KO mice than in DBA/1 mice. CIA in B6.IFNγ 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.IFNγ KO mice have a similar restricted TCR Vβ 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^q 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. IFNγ 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 IFNγ 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 IFNγ has a role in suppressing disease in CIA. The profound effect on susceptibility to CIA by disruption of the IFNγ pathway has been partially attributed to increased expression of IL12 and IL1β. However, IL12 and IL1β are proinflammatory cytokines shown to participate in promoting inflammation in CIA in mice with an intact IFNγ pathway. It is therefore not surprising that blocking either IL12 or IL1β can reduce the incidence and severity of arthritis in B6.IFNγ KO mice. As an alternative hypothesis, Matthys *et al* suggested that enhanced CIA in IFNγR KO DBA/1 mice might be related to CFA induced expansion of Mac1$^+$ myeloid cells. [24]

We have previously shown that IFNγ is involved in the elimination of activated CD4$^+$ T cells.[21 25] Failure to suppress the expansion of activated CD4$^+$ T cells in B6.IFNγ KO mice leads to a more severe and chronic progressive disease in experimental allergic encephalomyelitis.[21] IFNγ 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 IFNγ KO mice.[21] In DBA/1 IFNγR KO mice primed with CII in CFA, delayed-type hypersensitivity reactions to CII were enhanced,[24] which further supports an immunosuppressive activity of IFNγ 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 IFNγ, and subsequently the cells are eliminated from the joint or systemic circulation through IFNγ mediated apoptosis. It would be of interest to investigate whether there is a defect in the IFNγ pathway in CIA susceptible strains of mice such as DBA/1 and B10.Q mice.

The other explanation of arthritis susceptibility in these IFNγ KO mice is a dysregulation of chemokines in B6.IFNγ 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 IFNγR 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 Vβ6 or Vβ8 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.IFNγ 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] IFNγ 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.IFNγ 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 Vβ genes exert a dominant influence upon CIA susceptibility in mice. Using TCR Vβ congenic B10.Q (H-2^q) mice, Nabozny *et al* identified T cells using Vβ6 that contribute to arthritis in CIA.[5] A restricted TCR Vβ usage in arthritic joints in CIA has been demonstrated for both Vβ6 and Vβ8 in mice of H-2^q and H-2^r haplotypes.[2 4 19] In this study we demonstrate the predominant expression of Vβ6 in arthritic DBA/1 joints. Surprisingly, we found that B6.IFNγ KO (H-2^b) mice also showed a similar predominant expression of Vβ6 in arthritic joints. In addition, the Vβ8 family was the second most abundant Vβ phenotype expressed in arthritic joints from B6.IFNγ KO mice. In contrast, the TCR Vβ expression in draining lymph nodes from immunised mice showed no such restricted pattern, and there was no difference between TCR Vβ expression patterns in draining lymph nodes from CII immunised B6.IFNγ 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 IFNγ in B6 mice can occur.

Previous studies have identified the restricted T cell epitopes of the CII molecule in association with H-2^q.[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^q mice.[31] IFNγ is recognised to up regulate MHC molecule expression on antigen-presenting cells,[32] but it is not known whether IFNγ influences TCR-epitope-H-2 interaction. It will be important to compare T cell epitopes that are recognised by B6.IFNγ KO mice with those well characterised epitopes recognised by H-2^q and H-2^r mice.

Overall, susceptibility to CIA in B6.IFNγ 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^q DBA/1 mice.

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