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An anti-inflammatory role for interleukin-11 in established murine collagen-induced arthritis

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SUMMARY

Interleukin-11 (IL-11) is a cytokine belonging to the IL-6 family which has both pro- and anti-inflammatory potential. Like IL-6 it can diminish tumour necrosis factor-α and IL-1 production, and augment immunoglobulin synthesis. We have explored the immunomodulatory effects of IL-11 treatment in mice in a model of inflammatory autoimmune joint disease, collagen-induced arthritis (CIA). Recombinant human IL-11 was administered at various doses to DBA/1 mice after the onset of CIA. IL-11 treatment caused a significant reduction in the clinical severity of established CIA, which was associated with protection from joint damage, as assessed by histology. Although there was a suggestion at high doses of IL-11 that the anticollagen type II (CII) response may have been augmented, there was no statistically significant effect of IL-11 treatment on anti-CII antibody levels. Similarly, the acute-phase reactant serum amyloid P was only elevated in mice receiving very high doses (50–100 μg/day) of IL-11. Endogenous IL-11 was abundantly produced in synovial membrane cultures derived from CII-immunized mice with active disease, suggesting that, as in rheumatoid arthritis, this cytokine is spontaneously produced in the inflammatory response in CIA. The results presented here demonstrate an anti-arthritic immunoregulatory role for IL-11 in murine CIA, and suggest that IL-11 is a candidate therapeutic molecule for human inflammatory arthritic diseases.

INTRODUCTION

Interleukin-11 (IL-11) was initially identified as a stromal cell-derived lymphopoietic and haemopoietic factor,[1] but is now known to have pleiotropic activities in a wide range of haemopoietic and non-haematopoietic systems (reviewed in ref. 2). It supports the growth of plasmacytoma and hybridoma cells, acts with IL-3 to stimulate megakaryocyte colony formation by limiting the G0 period of stem cells, and supports megakaryocyte maturation and hence platelet production. In addition, IL-11 promotes erythropoiesis, enhances antigen-specific antibody responses, stimulates acute-phase protein synthesis, inhibits lipoprotein lipase activity and adipocyte differentiation, supports neuronal development and can induce cardiac muscle hypertrophy.[2] Many of the biological activities of IL-11 are shared with IL-6, leukaemia inhibitory factor (LIF), oncostatin M, ciliary neurotrophic factor and cardiotrophin-1. The overlapping functions of this group of cytokines can be explained, in part, by their common use of the gp130 transducing subunit in their high-affinity receptors, and the fact that some members also share the LIF receptor subunit (reviewed in ref. 3).

There is evidence that IL-11 may have an immunoregulatory role in joints affected by rheumatoid arthritis (RA). Like the immunomodulatory cytokine IL-10, there is abundant expression of IL-11 in RA synovial membrane cultures and neutralization of both cytokines produces a highly significant, synergistic increase in pro-inflammatory tumour necrosis factor-α (TNF-α) production.[4,5] Consistent with this hypothesis, IL-11 is produced by articular chondrocytes and synoviocytes which can then stimulate these cells to release tissue inhibitor of metalloproteinases (TIMP).[6] Furthermore, IL-11 can cause down-regulation of macrophage proinflammatory cytokine and nitric oxide production.[7]

Some properties of IL-11, however, are consistent with a possible detrimental role for the cytokine in RA. Notably, IL-11 can activate osteoclasts and thereby could promote bone resorption.[8–10] IL-11 can also enhance T-dependent antibody production both *in vitro* and *in vivo*,[11,12] therefore it may be important in the production of rheumatoid factors and other pathogenic antibodies. IL-11 can also stimulate production of some acute-phase proteins, which may help maintain homeostasis in inflammation, but their over-production may promote inflammatory processes.[2,13,14]

The experiments described here were aimed at discerning whether the net effect of exogenous IL-11 in a model of

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inflammatory autoimmune joint disease is pro- or anti-inflammatory. This was investigated by administration of recombinant human IL-11 to DBA/1 mice with established collagen-induced arthritis (CIA), a model which has been used extensively, by ourselves and others, to investigate the pathogenic mechanisms relevant to RA and to develop novel therapeutic approaches (reviewed in ref. 15). CIA is characterized by synovial hyperplasia and pannus formation resulting in extensive erosion of cartilage and bone.[16] As in RA, CIA can be ameliorated by the blockade of proinflammatory cytokines such as TNF-α and IL-1.[17–20] In this study systemic IL-11 treatment was found to cause a significant reduction in the clinical and histological severity of established CIA. The best therapeutic effect was achieved at a dose of IL-11, which did not adversely effect the anti-CII antibody response or elevate the acute-phase reactant, serum amyloid P. The combination of IL-10 and IL-11 did not improve the clinical outcome above therapy with the individual cytokines alone, but did reduce the level of joint damage.

MATERIALS AND METHODS

Therapeutic reagents

Recombinant human IL-11 was a gift from Dr G. Larsen and Dr S. Goldman (Genetics Institute, Inc., Andover, MA). The specific activity was $2{\cdot}1 \times 10^6$ U/mg.[21] Recombinant murine IL-10 was a gift from Dr S. Narula (Schering Plough, Nutley NJ). Both cytokines were diluted for administration in 0·1% bovine serum albumin (BSA), in endotoxin-free phosphate-buffered saline (PBS).

Induction and assessment of arthritis

Male DBA/1 mice (8–12 weeks old) were immunized by intradermal injection at the base of the tail with 200 μg of bovine CII, purified from bovine cartilage, as previously described.[22] Mice were inspected daily from 14 days after immunization for the onset of clinical disease and randomly assigned to a treatment group. The mice received daily doses of recombinant human IL-11 ranging from 0·3 to 100 μg (in a volume of 100 μl), or 100 μl of 0·1% BSA/PBS by intraperitoneal (i.p.) injection and were monitored for clinical score and paw swelling for 10 days. In experiments comparing or combining IL-10 treatment, mice were treated daily with 5 μg (in a volume of 100 μl) of IL-10 by i.p. injection for the same period. Paw swelling was assessed using callipers ('Pocotest', Kroeplin Langenmesstechnick, Germany) to measure the thickness of the first affected hind paw. For the clinical score, 0 = normal, 1 = slight swelling and/or erythema, 2 = pronounced oedematous swelling. Each paw was graded in this way giving a maximum possible score of 8 per mouse.

Histopathology

Paws were removed from mice post-mortem 10 days after disease onset and fixed in 10% buffered formalin and then decalcified in 5% ethylenediaminetetraacetic acid (EDTA) in buffered formalin. Paws were subsequently embedded in paraffin, sectioned sagitally and stained with haematoxylin and eosin (H & E) or safranin O. A histopathologist without knowledge of the treatment modality performed histological assessment. Arthritic changes in the distal interphalangeal (DIP), proximal interphalangeal (PIP) and metatarsophalangeal (MTP) joints were classified as mild, moderate, or severe. The following criteria were used: mild = minimal synovitis, cartilage loss and bone erosions limited to discrete foci; moderate = synovitis and erosions present but joint architecture intact; severe = synovitis, with extensive erosions and disrupted joint architecture. Since the bone resorption in arthritic joints of mice with CIA has osteoclastogenic origin, the osteoclasts, which have a distinct morphological form, were enumerated under × 100 magnification in the marginal zones of the joints. The mean number of osteoclasts/joint from each treatment group was determined.

Anti-collagen type II IgG responses

Serum anti-CII IgG levels at the termination of experiments were measured by a modification of an enzyme-linked immunosorbent assay (ELISA) described previously.[22] Briefly, microtitre plates (Immulon 2, Dynatech Laboratories, Bromborough, Wirral, UK) were coated with 2 μg/ml bovine CII, blocked and then incubated with serially diluted test sera. Bound IgG1 and IgG2a were detected by incubation with alkaline phosphatase-conjugated sheep anti-mouse IgG1 or IgG2a (Binding Site, Birmingham, UK), followed by substrate (*p*-nitrophenyl-phosphate). Absorbance was measured at 405 nm and compared to a standard of titrated, affinity-purified mouse anti-CII IgG.

Serum amyloid P levels

Serum amyloid P (SAP) levels were measured in terminal serum samples by ELISA, as described in ref. 23. Briefly, microtitre plates (Immulon 2, Dynatech Laboratories) were coated with 4 μg/ml trinitrophenylated keyhole limpet haemocyanin, before serial dilutions of sera and standard (murine SAP, Calbiochem-Novabiochem Ltd, Nottingham, UK) were added. Bound SAP was detected with rabbit anti-mouse SAP (Calbiochem-Novabiochem Ltd), followed by anti-rabbit IgG–alkaline phosphatase and *p*-nitrophenyl-phosphate. Absorbance was measured at 405 nm.

Endogenous IL-11 measurement

Synovial tissue was dissected from the knee joints of six individual mice, 26 or 51 days after immunization. The synovial tissue was then digested with 1 mg/ml collagenase A (Boehringer-Mannheim, Mannheim, Germany) and 150 μg/ml DNAse type IV (Sigma, Poole, UK) in the presence of 33 μg/ml polymyxin B sulphate (Sigma), then passed through a nylon sieve (Falcon, Becton Dickinson, Cowley, Oxford, UK) to form a single cell suspension as previously described.[24–26] Extensively washed cells were cultured at a density of 4×10^6/ml in a volume of 100 μl in flat-bottomed 96-well culture plates (Falcon, Becton Dickinson) in RPMI-1640 containing 10% (vol./vol.) fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2×10^{-5} M 2-mercaptoethanol and 1% (vol./vol.) glutamine. Supernatants were collected at 24 hr and analysed by ELISA. Capture and biotinylated detection antibodies were, respectively, 11hr3/19.6.1 and b11hr3/15.6.13 which were kindly provided by Dr G Larsen (Genetics Institute Inc., Andover, MA).

Equivalent reactivity of murine IL-11 in this ELISA has been confirmed at Genetics Institute, Inc.

Statistical analysis

For statistical analysis of macroscopic data the Mann–Whitney *U*-test of significance was applied. Comparison of histological data was made using Chi-squared analysis.

RESULTS

Amelioration of established arthritis by treatment with IL-11

Human IL-11 has comparable bioactivity to murine IL-11 on murine cells[27] and since insufficient quantities of murine IL-11 were available, arthritic mice were treated daily with human IL-11 initially with doses of 12, 25, 50, or 100 μg of IL-11 from disease onset. A profound suppression of both paw swelling and clinical score, compared with controls, was only found with the 50 or 100 μg doses of IL-11 (Fig. 1a). The significant reduction in both parameters of clinical assessment was not sustained however, for the whole of the 10-day observation period. The amelioration of arthritis was most pronounced from day 1 to day 7 of treatment, after which the effect diminished (Fig. 1a).

We considered it possible that the lack of sustained benefit of high-dose IL-11 therapy may be due to either immunogenicity or a net detrimental activity of the cytokine at high concentrations. Because of this possibility, and also because these quantities could not be realistically pursued in a human clinical setting, a lower dose regime was investigated. Doses of 0·3, 1, or 3 μg IL-11/day were administered from the day of clinical onset of arthritis (Fig. 1b). Both paw swelling and clinical score were found to be significantly reduced in mice treated with 3 μg daily doses of IL-11 at a number of time-points throughout the observation period. Groups treated with doses of 0·3 μg/day and 1 μg/day, however, showed no significant benefit compared with the controls.

Improvement in histological features of arthritis following IL-11 therapy

Histological analysis of joints from mice treated with high doses of IL-11 revealed that significantly more were mildly affected ($P<0·001$) and there were fewer severe joints ($P<0·001$), compared to controls (Fig. 2a). Similarly, joints from mice treated with 3 μg/day IL-11 also displayed more mildly affected ($P<0·001$) and fewer severe ($P<0·001$) joints (Fig. 2c). Consistent with the reduction of the extent of joint destruction there was also a significant reduction in the mean number of osteoclasts observed with both high-dose ($P<0·005$) (Fig. 2b) and low-dose regimes ($P<0·001$) (Fig. 2d).

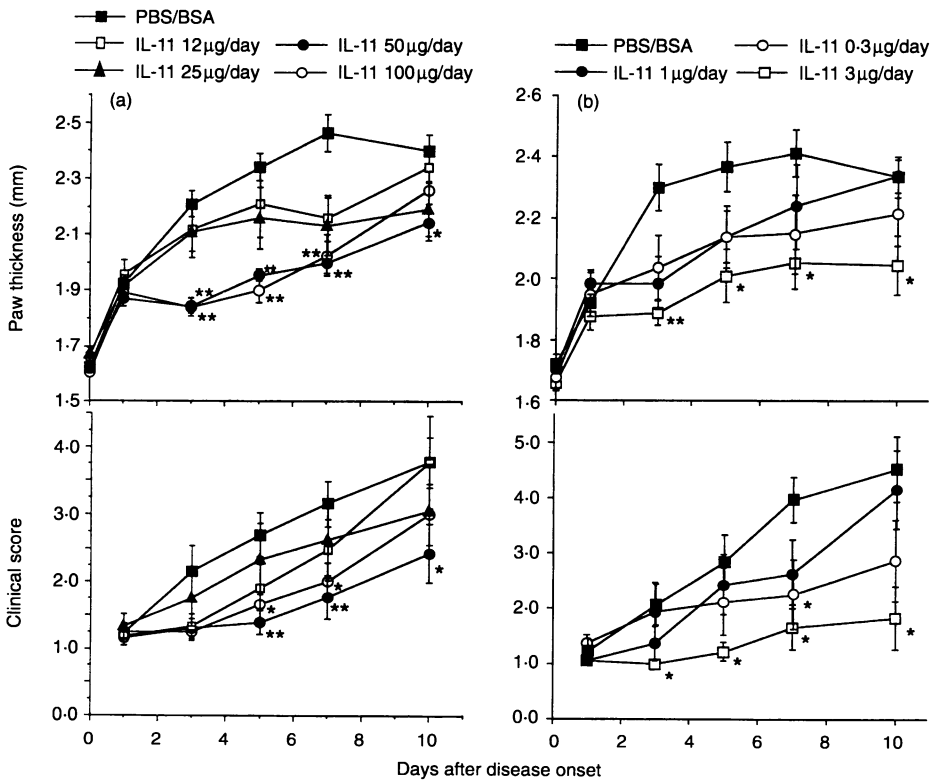

Figure 1. Effect of high- and low-dose IL-11 therapy on established arthritis. (a) Groups were treated with daily doses of IL-11 at 12 μg ($n=7$); 25 μg ($n=7$); 50 μg ($n=12$); 100 μg ($n=13$); or an equivalent volume of 0·1% BSA/PBS ($n=13$) for 10 days from the day of clinical onset. The top figure shows the change in paw thickness for the first affected hind limb. The lower panel shows the clinical scores. Values are the mean ± SEM; $*P<0·05$, $**P<0·005$. (b) Groups were treated with daily doses of IL-11 at 0·3 μg ($n=8$); 1 μg ($n=8$); 3 μg ($n=9$); or an equivalent volume of 0·1% BSA/PBS ($n=9$) for 10 days from the day of clinical onset. The top figure shows the change in paw thickness for the first affected hind limb. The lower panel shows the clinical scores. Values are the mean ± SEM; $*P<0·05$, $**P<0·005$.

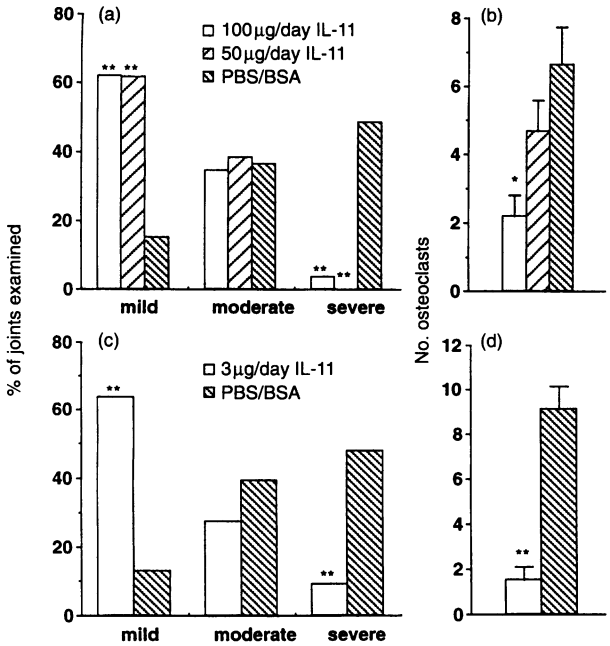

Figure 2. Effect of IL-11 treatment on histopathological severity of arthritis. Assessment was performed 10 days after the onset of disease and the commencement of treatment. (a) and (c) show the proportion of joints damage in the affected hind paws. (b) and (d) show the number of osteoclasts found in the joints. Number of paws/joints assessed: High-dose IL-11 treatment; 100 μg/day – 10/29, 50 μg/day – 13/34, PBS/BSA – 14/33; low-dose treatment; 3 μg/day – 8/22, PBS/BSA –8/23. $*P<0.01$; $**P<0.001$.

Lack of changes in anticollagen antibody responses following IL-11 therapy

Anti-CII IgG levels in the sera collected at day 10 of therapy were measured by ELISA. There appeared to be a trend towards a dose-dependent increase in the anti-CII IgG response following high doses of IL-11, but this was not statistically significantly different from the control group (Table 1). Low doses of IL-11 have previously been shown to augment the *in vivo* primary response to sheep red blood cells after only 7 days.[11] However, no significant differences in the anti-CII IgG1 and IgG2a responses were observed between treatment groups receiving 0·3–3 μg/day IL-11 and the control group (Table 1).

Augmentation of SAP production following high- but not low-dose IL-11 therapy

To determine whether IL-11 administration had affected the acute-phase response in CIA,[28] serum samples at the completion of clinical studies were assayed for SAP concentrations. The levels of SAP detected were significantly increased in mice treated with both 50 μg/day ($P<0.039$) and 100 μg/day ($P<0.021$) of IL-11, compared to controls (Fig. 3a). Treatments with 0·3–3 μg of IL-11 per day did not significantly alter serum levels of SAP (Fig. 3b).

IL-11 is present in synovial membrane cultures from arthritic mice

To determine the pattern of IL-11 production in inflamed joints, synovial membrane cultures were established from arthritic DBA/1 mouse joints and cytokine levels were measured. An ELISA with specificity for human IL-11 was used, as reagents for the specific detection of murine IL-11 were unavailable. A correlation was found to exist between IL-11 levels produced in synovial membrane cell cultures and the cellularity of the joint at day 26, and this was compared to clinical measurements of arthritis in the paws (Table 2). Three of six mice had synovial membrane cell counts in excess of 1×10^6, and were found to have levels of IL-11 in the range of 2·6–6·3 ng/ml. Two of those three mice had clinical arthritis as determined by fore- or hind-limb swelling and/or erythema. The third displayed no clinical signs of disease in the paws, despite hypercellularity in the knee joint. Synovia from mice taken at day 51 after immunization, in the remission phase of disease, showed hypercellularity in all of the joints dissected, but only one of the six samples tested was found to have significant levels of IL-11 (Table 2).

Combined treatment of arthritis with IL-10 and IL-11

Recent studies in our laboratory have shown that neutralization of both IL-10 and IL-11 in RA synovial membrane cultures produces a highly significant and synergistic increase in pro-inflammatory TNF-α production.[5] As the adminis-

Table 1. Anti-collagen IgG responses following IL-11 therapy*

Treatment	Anti-CII IgG (μg/ml) ± SEM	Anti-CII IgG1 (μg/ml) ± SEM	Anti-CII IgG2a (μg/ml) ± SEM
High-dose treatment			
IL-11 50 μg/day ($n=11$)	9926 ± 1060		
IL-11 100 μg/day ($n=11$)	10959 ± 1197		
PBS/BSA ($n=12$)	8033 ± 1135		
Low-dose treatment			
IL-11 0·3 μg/day ($n=8$)		1916 ± 428	8275 ± 2952
IL-11 1 μg/day ($n=8$)		3089 ± 1326	11919 ± 1655
IL-11 3 μg/day ($n=9$)		2028 ± 706	8215 ± 2009
PBS/BSA ($n=8$)		2053 ± 521	12154 ± 2789

*Anti-CII levels were assessed from serum collected at day 10 of treatment/disease onset by ELISA.

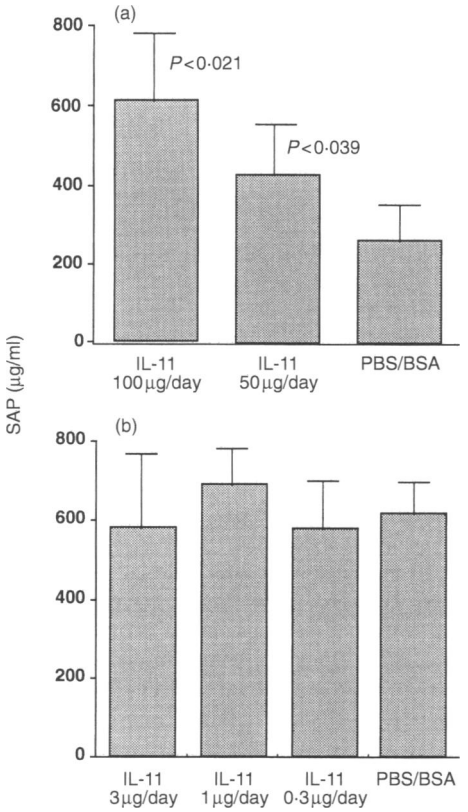

Figure 3. SAP responses following IL-11 therapy. SAP levels were assessed from sera collected at day 10 of treatment/disease onset by ELISA. (a) High-dose IL-11 therapy, IL-11 100 μg/day ($n=11$); IL-11 50 μg/day ($n=11$); PBS/BSA ($n=12$). Statistical significance of IL-11-treated mice compared with PBS/BSA treated controls is indicated. (b) Low-dose IL-11 therapy (separate experiment), IL-11 3 μg/day ($n=9$); IL-11 1 μg/day ($n=8$); IL-11 0·3 μg/day ($n=8$); PBS/BSA ($n=8$).

Table 2. Endogenous IL-11 production in synovial membrane cell cultures from CII immunised DBA/1 mice*

Knee joints sample no.	Clinical score	Total cell count (per knee × 10^6)	IL-11 (pg/ml)
26 days post-immunization			
1	2	2·80	3505
2	0	0·56	<300
3	2	1·31	2627
4	0	0·33	<300
5	0	1·54	6260
6	0	0·43	<300
51 days post-immunization			
1	0	1·54	<300
2	5	5·50	<300
3	6	4·78	<300
4	8	1·23	2725
5	0	1·28	<300
6	6	5·08	<300

*All cultures had a cell density of 4×10^6/ml.

tration of IL-10 ameliorates established CIA,[29] this treatment, in combination with IL-11, was explored in the CIA model. In two experiments, groups of mice were treated with daily doses of 3 μg IL-11, the previously determined effective dose of IL-10 of 5 μg/day,[29] a combination of the two cytokines at those doses, or an equivalent volume of 0·1% BSA/PBS. The results of the two experiments were pooled (Fig. 4).

Histological analysis of paws taken at the end of combination IL-11/IL-10 treatment showed a clear improvement over the other treatment groups and controls at day 10, with over 80% of the interphalangeal joints examined only mildly affected (Fig. 5). Consistent with the clinical results, there were less severely damaged joints and more minor joint changes in the mice that received IL-11, compared with controls (Fig. 6a). The level of joint protection for mice treated with IL-10 alone was consistent with that previously observed with a much larger sample[29] (Fig. 5).

As both IL-11 and IL-10 augment humoral responses,[11,30] sera from treated mice were assessed for anti-CII IgG1 and IgG2a production at day 10 of arthritis. As previously described[29] (Fig. 4), IL-11 or IL-10 treatment alone did not alter the levels of anti-CII IgG1 or IgG2a produced after 10 days of therapy (Fig. 6). The combined treatment with

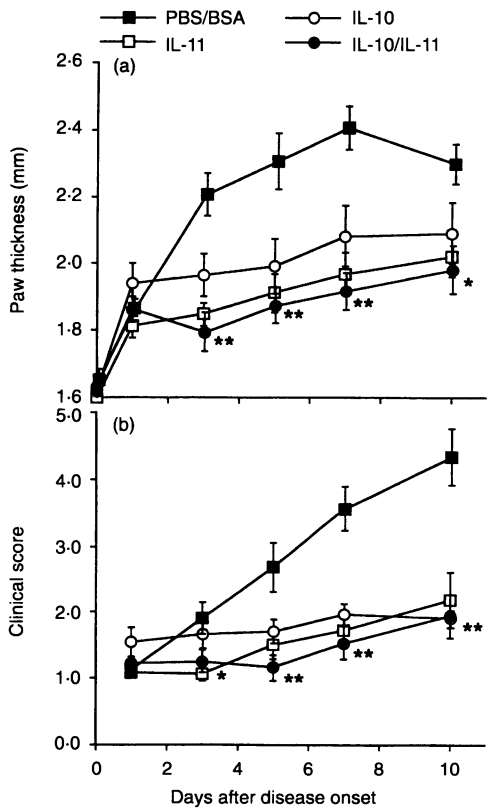

Figure 4. Effect of combined IL-11/IL-10 therapy on established arthritis. Pooled data from two concordant experiments. Groups were treated daily with IL-11 at 3 μg ($n=17$); IL-10 at 5 μg ($n=12$); a combination of IL-11 and IL-10 ($n=16$); or an equivalent volume of 0·1% BSA/PBS ($n=15$) for 10 days from the day of clinical onset. (a) Indicates paw thickness, (b) clinical score. Values are the mean ± SEM. *$P<0·05$, **$P<0·005$, for clarity, shown for combination therapy only.

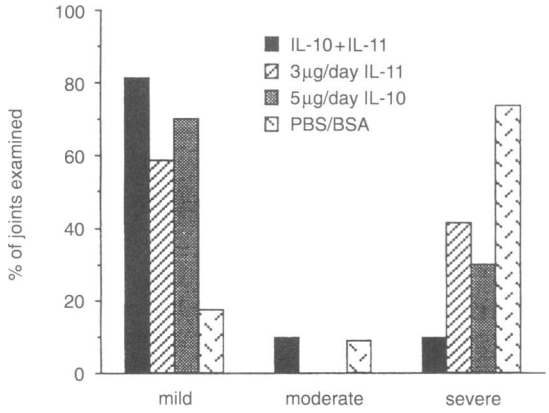

Figure 5. Histology following combined IL-11/IL-10 therapy. Histological assessment was performed 10 days after the onset of disease and the commencement of treatment. Number of paws/joints assessed: IL-11 3 μg/day + IL-10 5 μg/day – 8/21; IL-11 3 μg/day – 8/17; IL-10 5 μg/day – 4/10; PBS/BSA – 9/23.

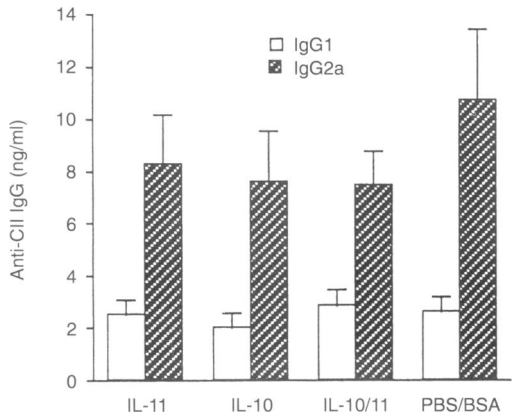

Figure 6. Anti-collagen IgG responses following combined IL-11/IL-10 therapy. Anti-CII IgG1 and IgG2a levels were assessed from serum collected at day 10 of treatment/disease onset by ELISA. IL-11 3 μg/day ($n=17$); IL-10 5 μg/day ($n=10$); combined IL-11/IL-10 treatment ($n=12$); BSA/PBS ($n=15$).

IL-11 and IL-10 also had no significant effect on anti-CII IgG1 and IgG2a levels.

DISCUSSION

We have shown here that the administration of IL-11 can ameliorate established CIA and, as such, it is acting as an anti-inflammatory cytokine, rather than a pro-inflammatory agent. This accords with *in vitro* studies from our laboratory on human RA synovial membrane cell cultures in which the blockade of IL-11 was found to raise TNF-α levels.[5] IL-11 was affective in ameliorating CIA at doses of 3, 50 and 100 μg/day. At the higher doses of 50–100 μg/day the beneficial affect of IL-11 was not sustained over the whole treatment course. Serum analysis showed that an anti-human IL-11 IgG response had developed (data not shown), thus the failure of the therapy in the latter part of the treatment programme is likely to be due to neutralization and/or rapid clearance of this human cytokine. Most unusually the intermediate doses of IL-11 (12 and 25 μg/day) had little effect on the clinical outcome of CIA. Why this occurs is not readily explainable. It may be that at these doses there is no net effect due to a balance between the pro- and anti-inflammatory actions of IL-11.[2,6–12]

The ameliorating effect of IL-11 administration on CIA is reminiscent of the effect of IL-10 on this disease.[29] IL-11 and IL-10 have been shown to inhibit the production of the pro-inflammatory cytokine TNF-α in rheumatoid synovial membrane cell cultures.[4,5] Both of these cytokines are expressed by cells from hyperplastic synovial tissue (Table 2 and refs. 25,26), but the level of expression seems to be insufficient to ameliorate disease as additional cytokine is effective. Studies on human synovial tissues have shown that there is a mutual effect of IL-11 and IL-10 on the production of each other, but they appear to have independent mechanisms for inhibiting TNF-α synthesis.[5] This may explain why there is no clinical benefit above what is achieved by one cytokine alone, for the mice receiving coadministration of IL-11 and IL-10. It could also be that the levels of IL-11 and IL-10 administered are too close to optimal and at lower doses synergy or additive effects may be revealed. Nevertheless, the combined IL-11 and IL-10 treatment at these doses has a protective effect on the joints, with less histological damage observed in the paws from these mice than from those treated with IL-11 or IL-10 alone. This dissociation between clinical improvement and joint protection has also been shown in mice with CIA treated with anti-IL-12 antibodies, in which there was a high degree of swelling but many of the joints were spared from destruction (Butler *et al.*, submitted for publication). It may be that with the combined treatment exogenously administered IL-11, in combination with the IL-10-stimulated endogenous IL-11, was able to further stimulate TIMP production and lower the MMP levels,[5,6] to give the observed joint protection.

Many of the pro-inflammatory or disease promoting actions of IL-11 were not observed with the CIA model. IL-11 has been described to increase bone resorption,[9] however, the joints from mice treated with high doses of IL-11 showed a less severe arthritis that contained fewer osteoclasts, than the controls. Regression analysis revealed a correlation between the number of osteoclasts and the histopathological severity observed (data not shown). There was inconclusive evidence that IL-11 administration augmented T-cell-dependent antibody responses,[11,12] as although there was a dose-dependent trend towards a higher anti-CII IgG response in mice treated with higher doses, this was not statistically significant. Serum levels of SAP, the murine acute-phase protein akin to C-reactive protein (CRP) in humans,[23] were elevated by treatment with high doses of IL-11, however, high levels of acute-phase proteins may serve to modify inflammation,[2] rather than enhance it. A similar anti-inflammatory mechanism involving acute-phase proteins has also been postulated for IL-6.[14] SAP levels were not elevated following treatment at the most therapeutically effective dose of 3 μg/day.

In summary, the data described here indicate an anti-inflammatory role for IL-11 in CIA both at the clinical and histological level. We also provide evidence that IL-11 is produced abundantly within the inflamed joints which suggests that, like IL-10, it is an endogenous component of the immunoregulatory response that occurs after acute pro-inflammatory provocation. The efficacy of IL-11 treatment in this model and in chronic inflammatory bowel disease in HLA-B27

transgenic rats[31] suggest that it is a candidate therapy for inflammatory arthritis, such as occurs in RA and in spondyloarthropathies.

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