Clin Exp Immunol 1994; 95:237–243

Amelioration of established murine collagen-induced arthritis with anti-IL-1 treatment

W. B. VAN DEN BERG, L. A. B. JOOSTEN, M. HELSEN & F. A. J. VAN DE LOO
Department of Rheumatology, University Hospital Nijmegen, Nijmegen, The Netherlands

(Accepted for publication 27 October 1993)

SUMMARY

Inflammatory cytokines have been implicated in the pathogenesis of rheumatoid arthritis. To validate a key role for IL-1 in arthritic processes we have studied the protective effect of neutralizing antimurine IL-1 antibodies in the murine collagen-induced arthritis (CIA) model. Combination of anti-IL-1α and anti-IL-1β given before onset of arthritis was shown to prevent disease completely. Remarkably, a single treatment was also highly effective in the established phase of arthritis, reducing both inflammation as well as cartilage destruction. Suppression was most pronounced with the combination, but anti-IL-1β alone also induced significant relief. Finally, we studied the protective effect of IL-1 neutralization on cartilage metabolism in a unilateral expression model of collagen arthritis. To this end zymosan was injected in one knee joint before onset of disease, resulting in accelerated expression in that particular joint and the draining paw. Anti-IL-1 treatment started after accelerated expression of arthritis was able to fully normalize chondrocyte synthetic function, which was highly suppressed in the control group. It is concluded that IL-1 is an important determinant in both inflammation and cartilage destruction in collagen arthritis, and this may have implications for therapy in human arthritis.

Keywords collagen arthritis IL-1 anti-IL-1 antibodies cartilage destruction

INTRODUCTION

There is increasing evidence to suggest that the cytokines IL-1 and tumour necrosis factor-alpha (TNF-α) play an important role in the pathogenesis of inflammatory joint diseases [1–3], including rheumatoid arthritis (RA). These cytokines have been identified in the synovial membrane and cartilage–pannus junction of arthritic joints from RA patients. Moreover, it has been suggested that a hierarchy exists in importance of cytokines, and TNF-α is claimed to be the driving force of other cytokines, including IL-1. Antibodies to TNF-α were shown to diminish the production of IL-1 by rheumatoid synovium-derived mononuclear cells [4]. Although TNF-α is able to mediate cartilage and bone destruction itself, IL-1 is more potent in this respect [5]. Numerous studies have shown the destructive effect of IL-1 *in vivo* upon injection in knee joints [6–8], whereas conclusive evidence for a similar direct role of TNF-α *in vivo* is lacking.

To prove the importance of TNF-α recent studies have addressed the effect of administering neutralizing anti-TNF antibodies to mice with collagen-induced arthritis (CIA). Such treatment was able to diminish onset of arthritis and also to ameliorate early collagen arthritis to a certain degree [9,10]. This provides evidence that TNF-α may be an important therapeutic target in RA. Meanwhile, we have shown that administering neutralizing antibodies against IL-1 can be very effective in reducing cartilage destruction in both murine antigen-induced arthritis and immune complex arthritis [11,12].

In the present study experiments are described aimed at validating the concept that IL-1 is a key element in the pathogenesis of arthritis and worth pursuing as a therapeutic target. Neutralizing antibodies against murine recombinant IL-1α,β were administered in various phases of murine collagen arthritis. Impressive reduction was noted both in inflammation as well as in cartilage destruction.

Correspondence: Wim B van den Berg PhD, Professor of Experimental Rheumatology, Dept of Rheumatology, University Hospital Nijmegen, 6500 HB Nijmegen, The Netherlands.

MATERIALS AND METHODS

Induction of arthritis

DBA/1j mice, aged 10–12 weeks at the start of the experiments, were immunized with bovine collagen type II (CII; 100 μg) emulsified in Freund's complete adjuvant (FCA; Difco, Detroit, MI), by four divided subcutaneous injections in the back. At day 21 the animals were boosted with an i.p injection of 100 μg collagen type II. Onset of polyarthritis occurred around 4–5 weeks. In some experiments the booster injection was omitted and the animals were given a local injection with 100 μg

Zymosan [13] in the right knee joint at day 28, to provoke expression of collagen arthritis in that particular joint and the draining paw.

Anti-IL-1 antibodies

Polyclonal rabbit antibodies were raised against murine recombinant, biologically active mature IL-1α and IL-1β, by immunization with FCA [11]. The recombinant IL-1 was generously provided by Ivan Otterness (Pfizer Central Research, Groton CT). Immunoglobulins were purified by salt precipitation and affinity chromatography on protein A Sepharose CL4B. The anti-IL-1α and anti-IL-1β antibodies showed no cross-reactivity against either IL-1β/IL-1α or a number of other cytokines such as IL-6, TNF and granulocyte-macrophage colony-stimulating factor (GM-CSF), and were roughly equivalent in potency. The antibodies have been demonstrated to show neutralizing capacity both *in vitro* and *in vivo*. Purified IgG (1 μg) is sufficient to neutralize completely the effect of 50–100 pg IL-1 in the NOB-1 bioassay. The antibodies have an excellent half life in mice (3 days), and were shown to neutralize both IL-1-induced cartilage proteoglycan synthesis inhibition *in vivo*, as well as *in situ* production and effects of IL-1 in the knee joint in two experimental murine arthritis models [11,12].

Treatment protocol and macroscopic scoring of arthritis

From day 24 after immunization mice were examined for macroscopic signs of arthritis in the paws, such as erythema and/or swelling. Treatment with antibodies was either started immediately or was delayed until arthritis was clearly established (see Results). Mice were always randomized between control and anti-IL-1 groups. In the case of treatment of established arthritis, groups were weighted for arthritic scores after randomization, to create balanced starting groups. Randomization was repeated when skew selection appeared.

Mice were scored at alternate days for erythema and swelling by two independent observers, resulting in mean scores with a maximum of 2 for each paw, and an overall maximum of 8 per animal.

Anti-IL-1 treatment of mice

Groups of mice were given a single i.p. injection of a standard dose of 1 mg purified IgG anti-IL-1α and 1 mg anti-IL-1β, unless stated otherwise. Control groups received 2 mg normal rabbit IgG, or purified IgG from rabbits immunized with the protein antigens lysozym or bovine serum albumin (BSA) in FCA. These latter treatments did not influence the course of collagen arthritis. In pilot experiments, doses of up to 3 mg of anti-IL-1 were given, with essentially similar results as obtained with 1 mg.

Histology

At the end of the experiments ankle and knee joints were dissected, fixed in formalin and processed for histology as described [13,14]. Standard frontal sections (7 μm) were prepared and stained with haematoxylin–eosin or Safranin O. Synovial infiltration and cartilage destruction were scored on five semi-serial sections of each specimen, spaced 10 sections apart, and mean values were obtained. Scoring was done in a blinded fashion by two observers.

Synovial infiltrate was graded on a scale of 0–3, according to the amount of inflammatory cells in the synovial tissue. Cartilage destruction was scored on a scale of 0–3, ranging from areas with dead chondrocytes (empty lacunae) or focal cartilage loss, loss of 1/4 to 1/2 of the cartilage, to complete loss of the articular cartilage. Collagen arthritis is a highly aggressive joint inflammation, which without treatment results in complete loss of articular cartilage in most of the animals.

Autoradiography

In one experiment animals were pulse labelled with ^{35}S-sulphate (50 μCi per animal) shortly (2 h) before sacrifice and knee and ankle joints prepared for histology. Sections were mounted on gelatin-coated slides, dipped in K5 emulsion (Ilford, Basildon, UK) and developed after 2–4 weeks [14].

Assessment of knee joint inflammation

Joint inflammation was quantified by ^{99m}Technetium pertechnetate uptake as described [11]. Briefly, mice were sedated by i.p. injection of 4·5% chloral hydrate, 0·1 ml/10 mg body weight. About 10 μCi ^{99m}Tc in 0·2 ml saline was injected subcutaneously in the neck region and accumulation of the isotope in the knee was determined after 30 min by external gamma counting. Inflammation is expressed as the ratio of Tc uptake in the right inflamed joint over the left non-inflamed joint. Values $>1·1$ were taken to indicate significant joint swelling.

Assessment of proteoglycan synthesis

Patellae were dissected from the knee joint, leaving the cartilage embedded in a minimal area of surrounding tissue. Specimens were cultured for 2 h in RPMI, containing 20 μCi ^{35}S-sulphate. Thereafter patellar cartilage was isolated and incorporation of ^{35}S-labelled proteoglycans (PG) quantified by liquid scintillation counting as described [15].

RESULTS

Early anti-IL-1 treatment

CIA develops in 70–80% of DBA/1j mice over a period of 70 days, with a gradual onset around 4–5 weeks. At day 25 mice were selected without macroscopic signs of arthritis. A single systemic treatment with anti-IL-1αβ antibodies was given at day 28 and development of arthritis was followed for 2 weeks. Within 42 days 5/11 mice of the control, normal IgG treated

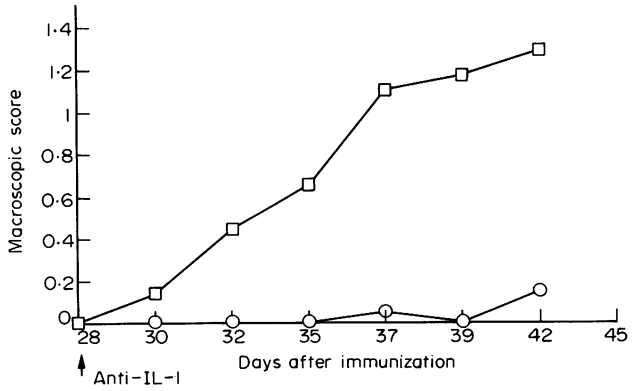

Fig. 1. Evolution of collagen arthritis. Mice were given a single i.p. injection of either 0·6 mg anti-IL-1α and 0·6 mg anti-IL-1β, or 1·2 mg normal rabbit IgG on day 28. This amount of antibodies has the capacity to neutralize completely the effect of 50 ng IL-1α/IL-1β in the NOB-1 bioassay (see Materials and Methods). Signs of arthritis were scored in all paws (scale 0–2) and values represent the mean of groups of 11 mice. ○, Anti-IL-1; □, normal IgG.

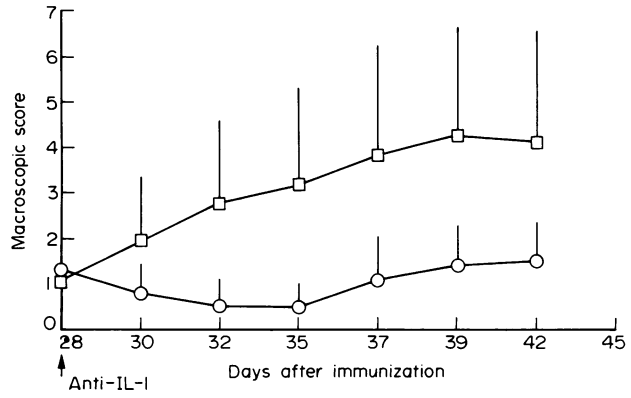

Fig. 2. Animals were selected with first signs of arthritis on day 25. Treatment and scoring as described in Fig. 1. Values represent the mean ±s.d. of groups of 10 mice. ○, Anti-IL-1; □, normal IgG.

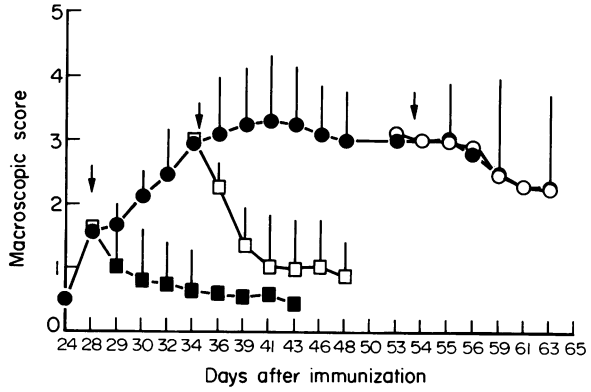

Fig. 3. Time course of collagen arthritis. A single treatment with anti-IL-1$\alpha\beta$ was given in separate groups at the time points indicated with arrows. Groups consist of 11, eight and six mice at the subsequent time points. For details see legend of Fig. 1. ●, Normal IgG; ■, anti-IL-1α, β; □, anti-IL-1α, β; ○, anti-IL-1α, β.

group developed significant but mild signs of arthritis. In contrast, the anti-IL-1 group remained free of arthritis till day 39 (Fig. 1). In repeat experiments we sometimes noted mild signs of arthritis at later days, but never a full blown but delayed expression of collagen arthritis. Similar amounts of antibodies against an irrelevant protein antigen (lysozym, BSA) were without effect.

Histology taken from the knee and ankle joints at day 42 confirmed our findings. In addition to the observations in the ankles, expression of arthritis was also clear-cut in the knee joints, with an incidence of 9/20 and 1/20 in control and anti-IL-1 groups, respectively.

From the same group of immunized mice, animals with clear first signs of arthritis were selected at day 25 and anti-IL-1$\alpha\beta$ treatment was given at day 28. Figure 2 shows the gradual rise in severity in the control group. This was prevented by anti-IL-1, although a tendency to rebound was noted after day 37. Histology at day 42 showed mean scores ±s.d. of synovial infiltrate in the knee of 1·2±1·1 and 0·3±0·6, respectively.

Suppression of established arthritis

In the next experiment mice with positive signs of arthritis were selected at day 25 and anti-IL-1$\alpha\beta$ treatment was given either at day 28, 35 or 54. Early treatment confirmed the rapid suppression of evolution as mentioned above. A remarkable and highly important finding was the clear decline of full blown arthritis, when treatment was delayed till day 35 (Fig. 3). In contrast, treatment started as late as day 54 was without effect.

Histological findings are depicted in Table 1. Anti-IL-1 antibodies were highly effective in reducing synovial infiltrate, both after early (day 28) as well as after late (day 35) treatment. Of interest, not only inflammatory signs but also the degree of cartilage destruction was reduced. The latter is a prominent feature of this form of arthritis, and generally shows a progressive, destructive character. Early treatment very effectively prevented cartilage destruction, but even treatment at day 35 significantly reduced ongoing destruction (Table 1).

Relevance of IL-1α and IL-1β

To investigate whether both IL-1α as well as IL-1β are involved, two experiments were done with separate sets of antibodies. No

Table 1. Histology after anti-IL-1 treatment

Group	Start of treatment* (day)	Day of sacrifice†	Joint	Synovial infiltrate‡	Positive joints§	Cartilage destruction
Control IgG	28	42	Ankle	1·2±0·8¶	16/22	0·7±0·7
Anti-IL-1$\alpha\beta$	28	42	Ankle	0·2±0·3**	6/22	0·1±0·2**
Control IgG	28	42	Knee	1·7±0·6	20/22	2·2±1·1
Anti-IL-1$\alpha\beta$	28	42	Knee	0·1±0·2**	8/22	0·5±0·9**
Control IgG	35	49	Ankle	1·1±0·8	11/16	1·9±1·3
Anti-IL-1$\alpha\beta$	35	49	Ankle	0·1±0·1**	7/16	1·1±1·2**

* Anti-IL-1 was given as a single injection at the day indicated.
† Histology was scored on total sections of whole knee or ankle joints.
‡ Synovial infiltrate and cartilage destruction are scored on a scale of 0–3 (see Materials and Methods).
§ Joints were considered positive when the intiltrate score was at least 0·1.
¶ Values represent the mean ±s.d.
** Significant difference ($P < 0·01$) between anti-IL-1 group and respective control (Mann–Whitney).

Table 2. Comparison of anti-IL-1α and anti-IL-1β treatment

Group	Start of treatment* (day)	Macroscopic arthritis score†	
		Day 34	Day 37
Experiment 1			
Control IgG	31	4·8 ± 1·2‡	4·6 ± 1·1
Anti-IL-1α	31	3·7 ± 2·3	3·6 ± 2·2
Anti-IL-1β	31	1·2 ± 1·4§	1·1 ± 1·3§
Anti-IL-1αβ	31	0·4 ± 0·3	0·3 ± 0·2§
		Day 40	Day 48
Experiment 2			
Control IgG	36	3·8 ± 2·3	4·0 ± 1·9
Anti-IL-1β	36	2·9 ± 1·0	2·5 ± 1·5
Anti-IL-1αβ	36	2·1 ± 1·4§	1·4 ± 1·0§

* Start of anti-IL-1 treatment.

† Macroscopic score in all paws.

‡ Values represent the mean ± s.d. of groups of 10 or seven mice in experiment 1 and 2, respectively.

§ Significant difference ($P < 0·01$) between anti-IL-1 group and IgG control (Mann–Whitney).

cross-reactivity was noted *in vitro* [11]. Suppression was most pronounced with the combination. Anti-IL-1α was hardly effective, but anti-IL-1β treatment induced highly significant suppression (Table 2). The decline was more pronounced when treatment was started early, but still impressive after late (day 36) treatment.

Zymosan accelerated arthritis

To reduce the high variation in degree of arthritis and to create consistent arthritis in all animals at a particular moment, we deleted the booster immunization with CII/Freund's incomplete adjuvant (FIA) at day 21. Instead, Zymosan (100 μg) was injected in the right knee joint around day 28. Zymosan induces transient joint inflammation with reversible joint damage, which wanes within 7 days in normal mice. However, it provokes expression of aggressive, long-lasting collagen arthritis in the injected knee joint of CII-preimmunized mice. In addition, it elicits within a few days expression of collagen arthritis in the draining paw only in most animals.

Systemic treatment with anti-IL-1αβ antibodies was given either shortly before, simultaneously or 2 days after Zymosan acceleration. Either treatment totally prevented the expression of arthritis in the ankle joint (Table 3). Moreover, knee joint inflammation was quantified by Tc uptake measurement and examined by histology. Anti-IL-1 treatment reduced Tc uptake at day 35 (7 days after Zymosan challenge) almost to background values (1·0). Reduction of inflammatory infiltrate was confirmed with histology (Table 3). Cartilage destruction was diminished from 1·5 ± 0·7 to 0·3 ± 0·3.

In addition, in one group of animals ^{35}S-sulphate was injected shortly (2 h) before sacrifice, to get an impression of the cartilage metabolism. Autoradiography on ankle and knee joint sections revealed highly suppressed ^{35}S-PG synthesis in the cartilage of the control, arthritic group, and almost normal incorporation in the cartilage of the anti-IL-1-treated group (Fig. 4).

Quantification of cartilage proteoglycan synthesis

In additional groups cartilage PG synthesis was quantified *ex vivo*. Patellae were isolated from the left, normal knee joint and the right, arthritic joint at day 7 after acceleration and pulse-labelled with ^{35}S-sulphate *in vitro*. Suppression of ^{35}S-PG synthesis amounted to approximately 60% (Table 4), which is consistent with observations in other arthritis models at phases of active joint inflammation [11,16]. In contrast, synthesis was normalized when anti-IL-1 was given simultaneously with the Zymosan acceleration. Strikingly, even an overshoot to markedly enhanced synthesis was found when anti-IL-1 was given 2 days after acceleration (Table 4). This points to an attempt at recovery of the depleted cartilage matrix, once the inflammatory process is controlled.

Table 3. Amelioration of Zymosan-accelerated arthritis

Group	Start of treatment* (day)	Arthritis at day 35†				
		Ankle		Knee		
		Score	Incidence	Tc uptake‡	Infiltrate§	Score ≥ 1¶
Control IgG	−1	1·0 ± 0·7	6/8	ND	ND	—
Anti-IL-1αβ	−1	0	0/10	ND	ND	—
Control IgG	0	1·4 ± 0·9	7/10	1·67 ± 0·20	2·5 ± 0·8	9/10
Anti-IL-1αβ	0	0	1/10	1·11 ± 0·12	0·7 ± 0·7	3/10
Control IgG	2	1·3 ± 0·8	8/10	1·68 ± 0·28	ND	—
Anti-IL-1αβ	2	0	0/10	1·08 ± 0·05	ND	—

* Relative to the injection of Zymosan at day 28.

† Arthritis is scored in the right knee and ankle.

‡ Expressed as a ratio of uptake in right inflamed *versus* left non-inflamed joint.

§ Synovial infiltrate on a scale of 0–3.

¶ Relative number of animals with a histological score ≥ 1.

Values represent the mean ± s.d.

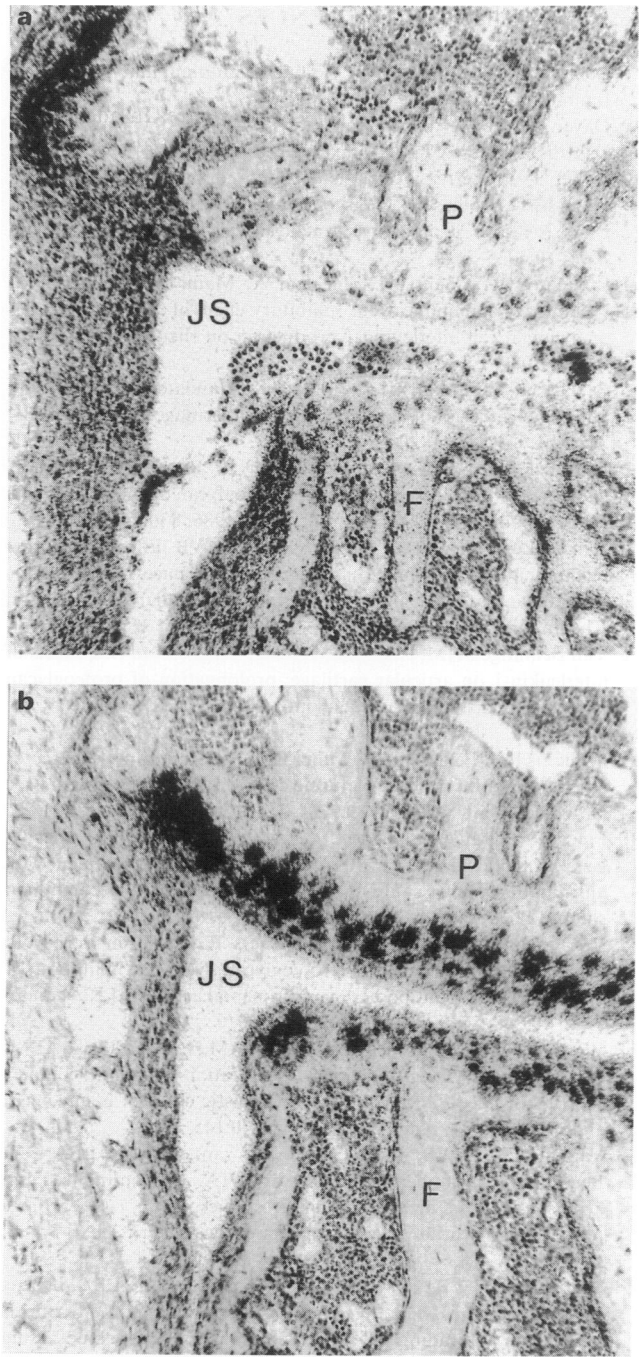

Fig. 4. Autoradiograph of a knee joint at day 35 after induction of collagen arthritis. (a) Arthritic control. (b) Anti-IL-1-treated. Note the faint labelling of chondrocytes in the arthritic joint and the distinct label incorporation in the anti-IL-1 treated joint. P, Patella; F, femur; JS, joint space. H&E staining.

DISCUSSION

The data indicate that IL-1 plays a critical role in murine CIA, not only at the onset of the disease but also in the more established phase. Apart from diminishing inflammation, the neutralization of IL-1 clearly reduced the progressive cartilage destruction in this model.

CIA is considered an important model for RA. It is based on the development of cross-reactive autoimmunity against homologous collagen type II, which is abundantly present in the hyaline articular cartilage. Both T cells and antibodies are claimed to be important in the pathogenesis. In recent years it was demonstrated that the expression of collagen arthritis could be highly enhanced by systemic administration of IL-1 [17,18]. Moreover, acceleration can be obtained using intraarticular injection of either TNF-α or transforming growth factor-beta (TGF-β) [19,20]. Our present results show that similar effects can be obtained with local injection of Zymosan, suggesting that any element causing cell influx and local generation of relevant cytokines would accelerate expression. In line with this it was shown that systemic lipopolysaccharide (LPS) could provoke expression of collagen arthritis [21]. We have confirmed this observation and elaborated the benefits of a localized, Zymosan-accelerated inflammation, with the interesting selective expression in the draining paw (manuscript in preparation).

There is increasing debate whether T cells are the driving force in the inflamed synovium of RA patients, or macrophages are the key elements. The latter is based on the minute analysis of cytokine patterns in inflamed synovia, and the observation of apparent lack of T cell lymphokines and abundance of macrophage cytokines such as TNF-α and IL-1 [22].

In any case, given the absence of a distinct T cell antigen in RA, it seems valid to approach these cytokines as therapeutic targets. There is suggestive evidence that TNF-α is regulating the production of IL-1 in rheumatoid synovia [4]. However, it seems a matter of taste whether the target should be chosen upstream. To our understanding it can not be ruled out that IL-1 is generated in part independently of TNF-α, and in that case a down-stream target would be more safe. We have recently performed studies with neutralizing MoAbs against murine TNF-α in the collagen model, and could confirm efficacy as demonstrated by others [9,10]. However, our present data with IL-1 neutralization are far more impressive. Proper dose response studies, tissue access and tuning of half lives of the antibodies are needed before definitive conclusions can be drawn.

The mechanism of onset of the inflammatory process in the joint in collagen arthritis is still poorly understood. It has been demonstrated that the disease can be transferred with CII-specific T cells [23], but the expression is greatly facilitated with antibodies. The latter is also noted in the autoimmune PG arthritis model [24]. It may be argued that the T cell-driven

Table 4. Cartilage proteoglycan (PG) synthesis in patellae of Zymosan-accelerated knee arthritis

Group	Start of treatment* (day)	Cartilage PG synthesis (day 35)		
		Left knee	Right knee†	%
Control IgG	0	793 ± 297‡	299 ± 67	38
Anti-IL-1αβ	0	861 ± 235	878 ± 243	102
Control IgG	2	849 ± 293	364 ± 224	43
Anti-IL-1αβ	2	859 ± 59	1704 ± 468	198

* Relative to the injection of Zymosan at day 28.

† Right knee injected with 100 μg Zymosan.

‡ Values represent ct/min ± s.d. of ^{35}S incorporated in patellar cartilage (groups of six mice).

process generates TNF and IL-1, and that these mediators potentiate the expression by their known destructive effect on the cartilage [4–8], with liberation of components including CII, and by their inflammatory effect, facilitating influx of antibodies [19] and attachment at the cartilage surface and further release of matrix components. This would fit with the ease of a single anti-IL-1 injection, to prevent onset of arthritis. The complete prevention with anti-IL-1 would also argue for an indirect role of TNF in this process. The sustained effect of a single injection at day 28 to prevent expression over a period of 2 weeks may be related to the phenomenon of anti-idiotypic down-regulation of threatening autoimmune responses, as seen in late phases of adjuvant arthritis [25]. It seems highly unlikely that the neutralization of IL-1 interferes directly with the antibody-dependent arm. We started anti-IL-1 treatment late in the immunization process (day 28) and could not detect any difference in anti-CII titres as measured with ELISA (data not shown). Similar lack of influence on antibody titres was noted in the anti-TNF studies [9,10].

The remarkable finding that collagen arthritis, established for 1 week, can still be effectively treated may point to another important IL-1-dependent mechanism. We have recently shown that synovial infiltrates rich in macrophages are highly susceptible to IL-1-induced flares [26], and similar findings were made in rat ankle joints bearing streptococcal cell wall driven chronic infiltrates [27]. This may imply that in the established phase of collagen arthritis part of the process is perpetuated by local IL-1 release and autocrine or paracrine stimulation of macrophages.

Another key finding was the protection against cartilage destruction. This may seem obvious given the marked reduction of inflammation, but on the other hand it is quite clear from drug studies in arthritis models that these two processes may occur uncoupled [28]. Earlier studies with neutralizing anti-IL-1 antibodies in antigen-induced arthritis and immune complex arthritis made it clear that IL-1 is a key factor causing cartilage PG synthesis inhibition, and this is now also confirmed in the collagen arthritis. It cannot be claimed that IL-1 is the only factor, but it implies that elimination of IL-1 from a network of cooperating mediators is sufficient to down-regulate this particular process.

In conclusion, this study underlines that it is worthwhile to pursue IL-1 as a therapeutic target in arthritis, not only in early phases but also in established arthritis. The application of neutralizing antibodies to human disease seems tempting, and the promising results with humanized anti-TNF antibodies may encourage such an approach [29]. On the other hand, other tools like IL-1 receptor antagonist (IL-1Ra) or soluble IL-1 receptors [2,30–33] may prove suitable in therapy. They suffer however from a short half-life and capture by IL-1Ra, respectively. In any case, our results strengthen the need for further search for improved IL-1 blockers or inhibitors of IL-1 production, and validation of the pathological importance of IL-1 in human RA by IL-1-targeted therapy.

ACKNOWLEDGMENTS

The generous gift of murine recombinant IL-1α and IL-1β by Ivan G. Otterness and Pfizer is gratefully acknowledged. The work was supported by the Dutch League against Rheumatism and the National Catholic Rheumatism Foundation.

REFERENCES

1 Brennan FM, Feldmann M. Cytokines in autoimmunity. Curr Opin Immunol 1992; **4**:754–9.
2 Dayer JM, Fenner H. The role of cytokines and their inhibitors in arthritis. Bailliere Clin Rheumatol 1992; **6**:485–516.
3 Keffer J, Probert L, Cazlaris H, Georgopoulos S, Kaslaris E, Kioussis D, Kollias G. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. EMBO J 1991; **10**:4025–31.
4 Brennan FM, Chantry D, Jackson A, Maini R, Feldmann M. Preliminary communication. Inhibitory effect of TNFα antibodies on synovial cell interleukin-1 production on rheumatoid arthritis. Lancet 1989; July 29, 244–7.
5 Saklatvala J. Tumour necrosis factor α stimulates resorption and inhibits synthesis of proteoglycan in cartilage. Nature 1986; **322**:547–9.
6 Pettipher ER, Higgs GA, Henderson B. Interleukin-1 induces leukocyte infiltration and cartilage proteoglycan degradation in the synovial joint. Proc. Natl Acad Sci USA 1986; **83**:8749–53.
7 Van de Loo AAJ, Arntz OJ, van den Berg WB. Effects of murine recombinant interleukin-1 on synovial joints in mice: measurements of patellar cartilage metabolism and joint inflammation. Ann Rheum Dis 1990; **49**:238–45.
8 Van Beuningen HM, Arntz OJ, van den Berg WB. *In vivo* effects of interleukin-1 on articular cartilage: prolongation of proteoglycan metabolism disturbance in old mice. Arthritis Rheum 1991; **34**: 606–15.
9 Williams RO, Feldmann M, Maini RN. Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. Proc Natl Acad Sci USA 1992; **89**:9784–8.
10 Piguet PF, Grau GE, Vesin C, Loetscher H, Gentz R, Lesslauer W. Evolution of collagen arthritis in mice is arrested by treatment with anti-tumour necrosis factor (TNF) antibody or a recombinant soluble TNF receptor. Immunol 1992; **77**:510–4.
11 Van de Loo AAJ, Arntz OJ, Otterness IG, van den Berg WB. Protection against cartilage proteoglycan synthesis inhibition by anti-interleukin 1 antibodies in experimental arthritis. J Rheumatol 1992; **19**:348–56.
12 Van Lent PLEM, van den Bersselaar LAM, van den Hoek AEM, van de Loo AAJ, van den Berg WB. Cationic immune complex arthritis in mice—A new model. Synergistic effect of complement and interleukin-1. Am J Pathol 1992; **140**:1451–61.
13 Van den Berg WB, Kruijsen MWM, van de Putte LBA, van Beusekom HJ, van der Sluis M, Zwarts WA. Antigen-induced and zymosan-induced arthritis in mice: studies on *in vivo* cartilage proteoglycan synthesis and chrondrocyte death. Brit J Exp Pathol 1981; **62**:308–16.
14 Van den Berg WB, van Beusekom HJ, van de Putte LBA, Zwarts WA, van der Sluis M. Antigen handling in antigen-induced arthritis in mice: an autoradiographic and immunofluorescence study using whole joint sections. Am J Pathol 1982; **108**:9–16.
15 Van den Berg WB, Kruijsen MWM, van de Putte LBA. The mouse patella assay: an easy method of quantitating articular cartilage chondrocyte function *in vivo* and *in vitro*. Rheumatol Int 1982;**1**: 165–9.
16 Kruijsen MWM, van den Berg WB, van de Putte LBA. Influence of the severity and duration of murine antigen-induced arthritis on cartilage proteoglycan synthesis and chondrocyte death. Arthritis Rheum 1985; **28**:813–9.
17 Killar LM, Dunn CJ. Interleukin-1 potentiates the development of collagen-induced arthritis in mice. Clin Sci 1989; **76**:535–8.
18 Hom JT, Bendele AM, Carlson DG. **In vivo** administration with IL-1 accelerates the development of collagen-induced arthritis in mice. J Immunol 1988; **141**:834–41.
19 Cooper WO, Fava RA, Gates CA, Cremer MA, Townes AS. Acceleration of onset of collagen-induced arthritis by intra-articular

injection of tumour necrosis factor or transforming growth factor-beta. Clin Exp Immunol 1992; **89**:244–50.

20 Thorbecke GJ, Shah R, Leu CH, Kuruvilla AP, Hardison AM, Palladino MA. Involvement of endogenous tumor necrosis factor α and transforming growth factor β during induction of collagen type II arthritis in mice. Proc Natl Acad Sci USA 1992; **89**:7375–9.

21 Caccese RG, Zimmerman JL, Carlson RP. Bacterial lipopolysaccharide potentiates type II collagen-induced arthritis in mice. Mediators of Inflammation 1992; **1**:273–9.

22 Alvaro-Garcia JM, Zvaifler NJ, Brown CB, Kaushansky K, Firestein GS. Cytokines in chronic inflammatory arthritis. J Immunol 1991; **146**:3365–71.

23 Brahn E, Trentham DE. Experimental synovitis induced by collagen specific T cell lines. Cell Immunol 1989; **118**:491.

24 Mickecz K, Glant TT, Poole AR. Immunity to cartilage proteoglycans in BALB/c mice with progressive polyarthritis and ankylosing spondylitis induced by injection of human cartilage proteoglycan. Arthritis Rheum 1987; **30**:306–18.

25 Cohen IR, Weiner HL. T cell vaccination. Immunol Today 1988; **9**:332.

26 Van de Loo AAJ, Arntz OJ, van den Berg WB. Flare-up of experimental arthritis in mice with murine recombinant IL-1. Clin Exp Immunol 1992; **87**:196–202.

27 Stimpson SA, Dalldorf FG, Otterness IG, Schwab JH. Exacerbation of arthritis by IL-1 in rat joints previously injured by peptidoglycan-polysaccharide. J Immunol 1988; **140**:2964–9.

28 De Vries BJ, van den Berg WB. Impact of NSAIDs on murine antigen induced arthritis. I. An investigation of antiinflammatory and chondroprotective effects. J Rheumatol 1989; **Suppl 18, 16**:10–8.

29 Elliot MJ, Maini RN, Feldmann M, Charles P. Treatment of rheumatoid arthritis with chimaeric monoclonal antibodies to TNF α. Safety, clinical efficacy and control of the acute-phase response. Clin Rheumatol 1993; **12**:34.

30 Hannum CH, Wilcox CJ, Arend WP *et al.* Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. Nature 1990; **343**:336–40.

31 McIntyre KW, Stepan GJ, Kolinsky KD *et al.* Inhibition of interleukin-1 (IL-1) binding and bioactivity *in vitro* and modulation of acute inflammation *in vivo* by IL-1 receptor antagonist and anti-IL-1 receptor monoclonal antibody. J Exp Med 1991; **173**:931–9.

32 Schwab JH, Anderle SK, Brown RR, Dalldorf FG, Thompson RC. Pro- and anti-inflammatory roles of interleukin-1 in recurrence of bacterial cell wall-induced arthritis in rats. Infect Immun 1991; **59**:4436–42.

33 Arend WP. Interleukins and arthritis. IL-1 antagonism in inflammatory arthritis. Lancet 1993; **341**:155–6.