TNFα and IL-1β are separate targets in chronic arthritis

W.B. van den Berg, L.A.B. Joosten, F.A.J. van de Loo

Department of Rheumatology, University Hospital Nijmegen, The Netherlands. Wim B. van den Berg, PhD, Prof. Exp. Rheumatology; Leo A.B. Joosten, MSc; Fons A.J. van de Loo, PhD.

Please address correspondence and reprint requests to: Prof. Wim B. van den Berg, Department of Rheumatology, University Hospital Nijmegen, Geert Grooteplein Zuid 8, 6500 HB Nijmegen, The Netherlands. E-mail: w.vandenberg@reuma.azn.nl

Clin Exp Rheumatol 1999; 17 (Suppl. 18): S105 - S114.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 1999.

Key words: Cartilage destruction, chronic inflammation, anti-cytokine therapy.

ABSTRACT

Chronic arthritis is characterized by persistent joint inflammation and concomitant joint destruction. Using murine arthritis models and neutralizing antibodies as well as cytokine-specific knockout conditions, it was found that tumor necrosis factor α (TNFα) is important in joint swelling, whereas a direct role in tissue destruction is unlikely. Interleukin-1 (IL-1) is not a dominant cytokine in early joint swelling, but has a pivotal role in sustained cell infiltration and erosive cartilage damage. TNFα-independent IL-1 production is a prominent feature in murine arthritis models, implying that IL-1 as well as TNFα are appropriate targets for therapy. These observations provide evidence for the potential uncoupling of joint inflammation and erosive changes and underline the need for TNFα /IL-1 directed combination-therapy approaches.

Introduction

Rheumatoid arthritis (RA) is characterized by chronic inflammation of multiple joints and marked destruction of cartilage and bone. The pathogenesis is largely unknown, but RA is generally considered to be an autoimmune disease, with primary or secondary involvement of T cells. However, particular (auto)-antigens have not been identified yet, thus hampering specific immunomodulation as a therapeutic approach.

Apart from T cell involvement, evidence is accumulating that the inflammatory process in the synovial tissue is dominated by activated monocytes and fibroblasts (1). Cytokines and cytokine inhibitors derived from these cell types can be abundantly found, and dominant cytokines do provide another, approachable target in this disease. It is now commonly accepted that tumor necrosis factor α (TNFα) and interleukin-1 (IL-1) are pivotal mediators in the RA process (2, 3). Clinical trials have revealed that TNF neutralization provides substantial relief of RA symptoms, such as pain and the number of swollen joints. So far it is unclear whether anti-TNFα therapy also reduces cartilage and bone erosions. Although IL-1 blocking with IL-1 reactor antagonist (IL-1ra) did not yield a clear improvement of clinical symptoms, data from the first clinical trial (4) provided evidence for an amelioration of joint erosions, which suggests that IL-1 may be a crucial target as well.

Given the poor regenerative capacity of cartilage, it is ethically inappropriate to take cartilage biopsies in the early stages of human inflammatory arthritis. This situation seriously hampers proper evaluation of the efficacy of anti-cytokine therapy on erosive processes in RA patients. Detailed studies in animal models of arthritis are therefore of crucial importance to the further understanding of the mechanisms underlying cartilage destruction, and the identification of feasible modalities of cartilage and bone protective therapy. In the following sections, emphasis will be given to recent findings regarding the cytokine interplay in murine arthritis models, with particular attention to be focused on the distinct roles of TNFα and IL-1β. Apart from the inflammatory process in the joint, major attention is given to aspects of cartilage and bone destruction.

Cartilage-destructive cytokines

Articular cartilage is an acellular tissue consisting of chondrocytes embedded in a highly structured matrix. Proteoglycans (PG) and collagen type II are the major macromolecules in this tissue, providing tensile strength and load-bearing capacity. Cartilage destruction in chronic arthritis is characterized by two major events: the reduced synthesis of matrix components by articular chondrocytes, and the enhanced breakdown of cartilage matrix by enzymes originating from the cartilage itself and/or from the inflamed synovial compartments.

Early changes identified in experimental arthritis models include PG loss with concomitant denuding of the tight collagen meshwork. This event makes the matrix vulnerable to further damage. A

major step in the loss of articular cartilage in erosive tissue is the destruction of collagen bundles. Apart from enzymes released from synovial cells, the enhanced breakdown of cartilage may result from the cytokine activation of chondrocytes.

When the presently identified cytokines are considered, it is clear that both TNFα and IL-1 can cause cartilage damage *in vitro*, but IL-1 is much more potent, with proven efficacy *in vivo* (5). In principle, no difference in potency has been noted between the two isoforms IL-1α and IL-1β. It is believed that IL-1α is a major cytokine in the early stages of inflammation, whereas IL-1β is the more dominant cytokine in advanced inflammation. Although TNFα is far less potent, it is noteworthy that TNF may display considerable synergism with IL-1 (6). More recently, IL-17 and Oncostatin M have been identified as cartilage-destructive cytokines. Their relative potency and potential synergism with the IL-1/TNFα system have yet to be determined.

Apart from initial studies involving the local, intraarticular injection of recombinant forms of IL-1 (5), overexpression of IL-1 has now been achieved by local gene transfer in the rabbit knee joint, demonstrating a full blown-arthritis (7). In addition, transgenic mice overexpressing IL-1α also display full signs of a destructive inflammatory arthritis (8). Both studies emphasize that IL-1 alone can elicit the major manifestations of arthritis. Intriguingly, it was shown by elegant studies in TNF-expressing transgenic mice that TNFα overexpression leads to chronic arthritis, yet treatment with antibodies to the IL-1 receptor fully abolished the arthritic process (9). Remarkably, TNF levels were still high in these animals, excluding a direct destructive effect of TNFα alone *in vivo* and further substantiating the predominant role of IL-1.

Murine models of arthritis

Although the above data illustrate the potency of cytokines in single-mediator systems, their actual roles remain to be identified in accepted arthritis models, using the approach of neutralising antibodies, soluble receptors, cytokine-specific knockout mice, and others. In line with current thinking on the RA process, the models should preferably reflect conditions of TNFα-dependent joint inflammation driven by T cells or macrophages.

In the present paper, studies will be described involving two types of models: allergic joint inflammation directed against protein (auto)antigens in the joint and driven by T cells and immune complexes; and macrophage-driven disease caused by local activation in the joint with phlogistic stimuli such as persistent bacterial cell wall fragments.

The most widely studied model in inflammatory arthritis research is autoimmune collagen-induced arthritis (CIA), which is based on immunization with foreign collagen type II in genetically susceptible mice, primarily DBA/1j or B10RIII mice. This model exemplifies an autoimmune process against an autoantigen in the articular cartilage. Arthritis expression is dependent upon a mixture of anti-collagen type II antibodies and anti-CII T-helper type 1 cells (Th1), and the polyarthritis starts around 4 weeks after the first immunization against collagen type II, usually in a limited number of joints with gradual spreading to multiple joints. The expression can be facilitated by the systemic injection of TNF/IL-1 or the indirect generation of such mediators by the induction of non-specific inflammatory events around the time of expected onset (10, 11).

Role of TNFα and IL-1 in collagen arthritis

When anti-TNFα antibodies or other TNFα inhibitors were given before or shortly after the onset of the first signs of arthritis, marked amelioration of joint inflammation was demonstrated by various groups, mainly analysed by the scoring of inflammatory signs in the paws (12, 13). This is encouraging, since signs of inflammation in human RA showed TNFα dependence as well. We have compared the efficacy of TNFα and IL-1 blocking in this model, including a kinetic study starting anti-cytokine treatment at various days after the onset of arthritis. It confirmed that TNF blocking is effective in the early stages. However, less efficacy was noted in established disease (Fig. 1).

In contrast, the suppressive effect of IL-1 elimination was more pronounced and was also prominent in established arthritis (14, 15). IL-1β appeared to be more important than IL-1α, since selective blocking with antibodies to IL-1β was almost as effective as blocking with anti-IL-1α + anti-IL-1β.

The role of IL-1 was further substantiated with IL-1ra treatment. Initial attempts to block collagen arthritis with

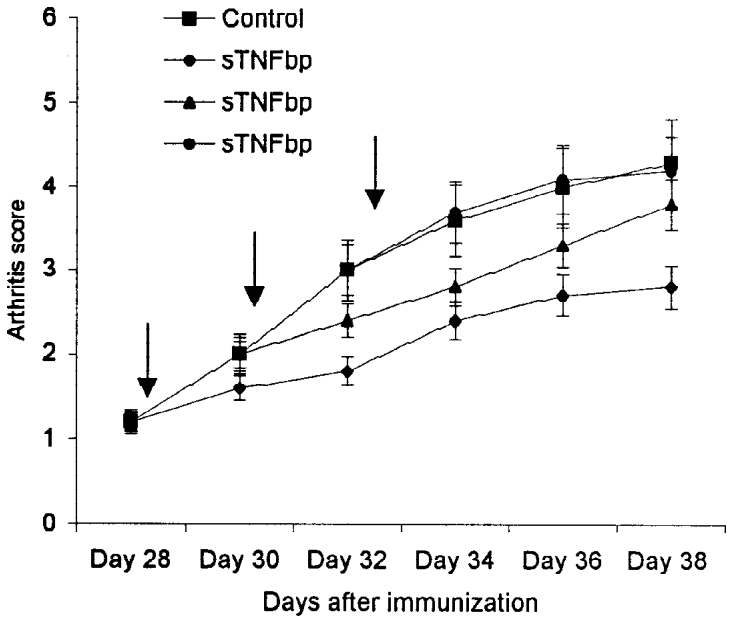

Fig. 1. Treatment of collagen arthritis with tumor necrosis factor (TNF) binding protein, starting at various days after the onset of arthritis (**arrows**). Note the loss of effect in established disease.

repeated injections of high doses of IL-1ra were unsuccessful in our hands. IL-1ra is known for its poor pharmacokinetics and appeared highly effective only when applied with osmotic minipumps (14, 15). Under conditions of high, sustained dosing, a continued IL-1 receptor occupancy could be achieved. Sustained treatment with IL-1ra not only abolished the inflammation, but also fully normalized the suppressed chondrocyte synthetic activity (14). Recently, we confirmed the efficacy of IL-1ra in this model using local IL-1ra gene transfer with a retroviral system and murine fibroblasts as a carrier (16).

The strong IL-1 dependence of this model is further substantiated by its reduced expression in IL-1β-converting enzyme (ICE) knock-out mice and the efficacy of ICE inhibitors in the CIA in normal mice (17). Moreover, full prevention of CIA is seen in IL-1β knock-out mice (unpublished observation). Similar approaches with such immune-driven models in TNF deficient-mice are hampered by the fact that these mice show major abnormalities in lymphoid tissue organization and the generation of immune responses (18). An elegant alternative is provided by the recent studies in TNF-receptor 1-deficient mice crossed with DBA/1 mice. Upon immunization with CII, these mice developed CIA with a lower incidence and in a milder form. However, once a joint was afflicted, the arthritis progressed in that joint to the same end stage as that observed in the wild-type mice (19). This again argues that TNFα is important at onset, but that progression is TNFα-independent. Along the same lines, it may suggest that anti-TNF treatment in RA patients may be beneficial when the chronicity of the disease is in fact based on repeated acute episodes, with each flare displaying TNF dependency.

When comparing the efficacy reported by various laboratories of anti-TNFα treatment in this model, marked variation is noted. This is probably related to the variable susceptibility to and expression of disease in DBA/1j mice in the different animal facilities, differences in the stringency of the immunization protocol to break natural tolerance to collagen type II, and the quality of the anti-TNFα inhibitors. In our hands, it becomes clear that higher and more prolonged TNFα dependence is found when the model is not accelerated by non-specific stimuli such as lipopolysaccharide (LPS). The latter will synchronize the arthritis expression in multiple joints and create advanced disease in almost all joints within a week after onset. When spontaneous, non-accelerated expression is analysed, it is obvious that such a protocol will allow initiation of the arthritis in some joints, with slow spreading to other joints over a period of weeks. In fact, every new onset in a naive joint is highly TNFα-dependent, and cumulative scores will show some TNFα dependency at various weeks after onset of the first signs.

Cartilage destruction in collagen arthritis with anti-TNFα?

When histology of joints is analysed after treatment with either anti-TNFα or anti-IL-1, it becomes clear that anti-TNFα provides protection against cartilage destruction only when treatment is initiated at the onset of collagen exposure. In fact, it is only protective when expression of the destructive autoimmune process can be prevented. If treatment with anti-TNFα is started shortly after onset, swelling reduction is still seen, but histology reveals that the reduction of cell influx in the synovium is marginal and cartilage damage is unaffected.

Apart from proper histologic evaluation, we recently screened the levels of cartilage oligomeric matrix protein (COMP) (20) in the circulation of mice with CIA, after treatment with either anti-TNFα or anti-IL-1. COMP is a marker of the enhanced turnover of articular cartilage; levels are markedly elevated in CIA and correlations are found with the degree of cartilage damage in non-treated mice (Fig. 2A). When anti-TNFα treatment was initiated after the onset of arthritis, we did not observe a reduction in the elevated COMP levels (Fig. 2B), and this

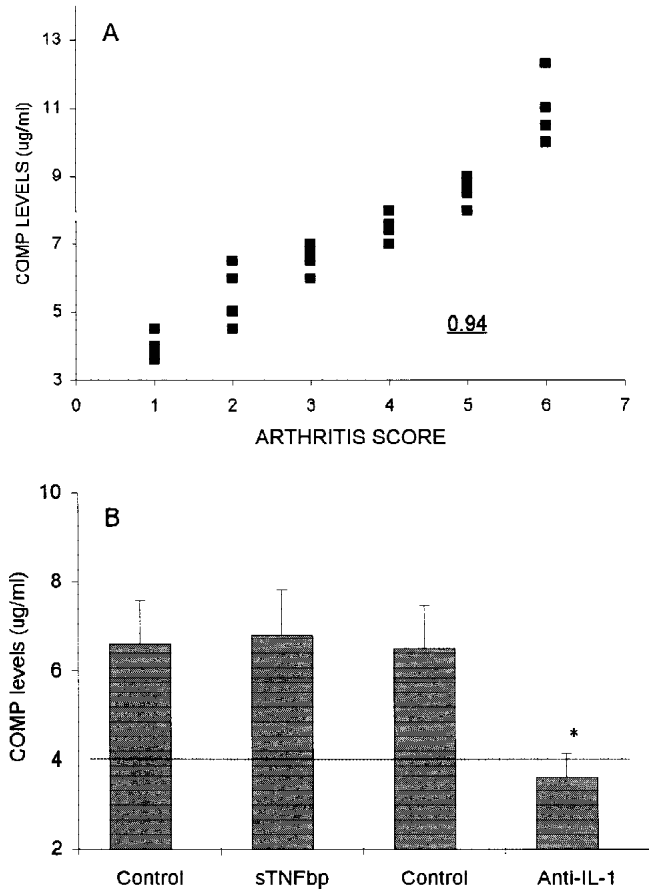

Fig. 2. Correlation between COMP (cartilage oligomeric matrix protein) levels in the serum at day 38 with the severity of arthritis at that moment (**A**). After treatment with TNFbp no reduction is seen, whereas reduction to normal levels (**line**) is observed after anti-IL-1α,β treatment (**B**).

correlated with the lack of protection against cartilage damage on histologic examination. In clear contrast, anti-IL-1 treatment up to 36 days after exposure to collagen normalized COMP levels to the values found in non-arthritic mice. This further underlines the great differences in the impact of TNFα and IL-1 neutralization on cartilage destruction in this model (20).

TNFα/IL-1 involvement in SCW arthritis

The above data about collagen arthritis suggest that not all IL-1 is driven by TNF, since IL-1 blocking was more efficacious than TNF blocking, particularly in late-stage CIA. This would suggest that anti-TNFα therapy is not sufficient to control inflammation as completely as possible in RA, and that combination therapy with anti-IL-1 should be mandatory. However, it can still be argued that the TNF blocking was not optimal in these experimental models, or that advanced stages of the collagen arthritis model do not reflect the human disease. The first argument appears unlikely since studies were performed with high doses of neutralising antibodies as well as soluble TNF binding proteins. The second argument, that RA is a disease process with triggering elements different from CIA, can of course never be excluded.

To get a better impression of the general validity of the need for separate TNFα and IL-1 blocking, we focused our further studies on the involvement of these cytokines in the model of streptococcal cell wall (SCW)-induced arthritis. Bacterial cell walls are potent inducers of TNFα, and in that sense the cytokine pattern in SCW arthritis, after intra-articular injection of cell wall fragments in the murine knee joint, mirrors closely the clinical situation in RA patients. Since differences between the *in vivo* potency of neutralising antibodies or scavenging receptors against TNFα and IL-1 can never be fully excluded, we performed additional studies in TNFα and IL-1β knock-out mice.

In this model, pronounced uncoupling between mediator involvement in joint swelling, synovial infiltrate, and cartilage damage is seen. When TNFα is blocked, using either anti-TNF antibodies or TNF binding protein (engineered soluble receptor from Amgen), a marked dose-dependent reduction in joint swelling is found, once again underlining the crucial role of TNFα in this parameter (Fig. 3). In marked contrast, the inhibition of chondrocyte PG synthesis in articular cartilage, which is a key event in the articular cartilage in all types of joint inflammation and which is clearly an IL-1-dependent phenomenon, remained unchanged. Of note, twice-daily systemic injections with IL-10 mimicked the anti-TNFα effect, in that it reduced joint swelling but did not normalize the inhibition of synthesis in articular cartilage. This is compatible with the findings that IL-10 is a powerful TNFα inhibitor but has marginal effects on IL-1 production *in vivo* (21).

In sharp contrast, IL-1 blocking with neutralising antibodies or IL-1ra did not reduce joint swelling, but fully normalized chondrocyte synthetic activity (22). Intriguingly, and compatible with its ongoing impact on cartilage, TNFα blocking *in vivo* did not significantly reduce IL-1 levels in arthritic synovial tissue (22). Histology at day 7 made it clear that anti-TNFα treatment did not protect against PG loss from the articular cartilage, whereas this protection was impressive after IL-1 blocking. Combined blocking with anti-TNFα and anti-IL-1 clearly provided the best overall protection, as both the initial swelling and the cartilage damage at day 7 were optimally controlled. However, protection against cartilage damage was not improved as compared to that achieved with anti-IL-1 alone (22), again underlining that TNFα and IL-1 have separate activities.

SCW arthritis in TNFα and IL-1β knock-out mice

To substantiate the uncoupling of mediator involvement in swelling and cartilage metabolism, and to exclude the possibility that the observed TNFα independence of IL-1 production and action were not due to inadequate TNFα blocking, we also studied SCW arthritis in TNFα and IL-1β knock-out mice. Since SCW arthritis is not an immune-driven model, the immunologic impairment of TNFα knock-out mice does not limit the analysis of this model. The IL-1β knock-out mouse is not impaired and shows a normal phenotype, with good delayed hypersensitivity responses to protein antigens, as well as normal LPS-induced inflammation, with expected levels of circulating IL-1, IL-6, and TNF (23).

When SCW arthritis was induced in TNFα knock-outs, joint swelling was fully reduced and late synovial infiltrate (day 7) was markedly reduced, but cartilage PG loss was barely diminished (Fig. 4), observations which are in line with data obtained with TNFα blocking.

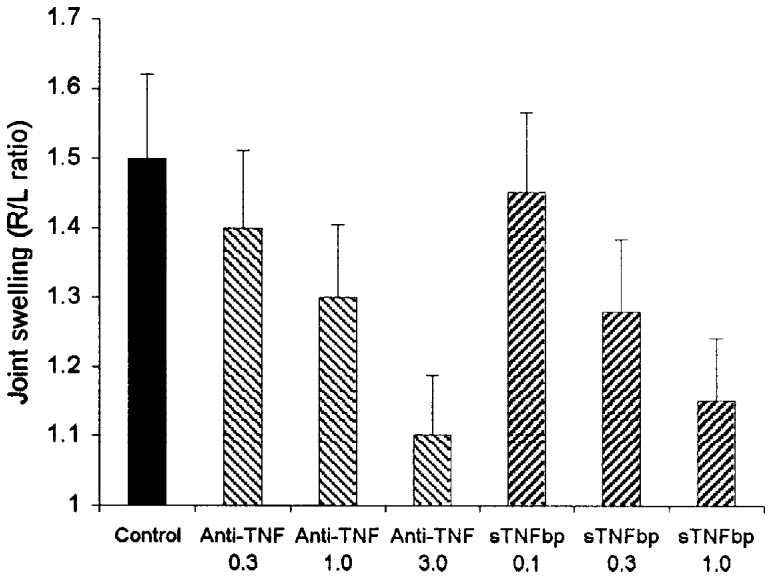

Fig. 3. Dose-dependent suppression of joint swelling with anti-TNFα or TNFbp. Treatment was started shortly before the induction of arthritis, and measurements of joint swelling were made at day 2.

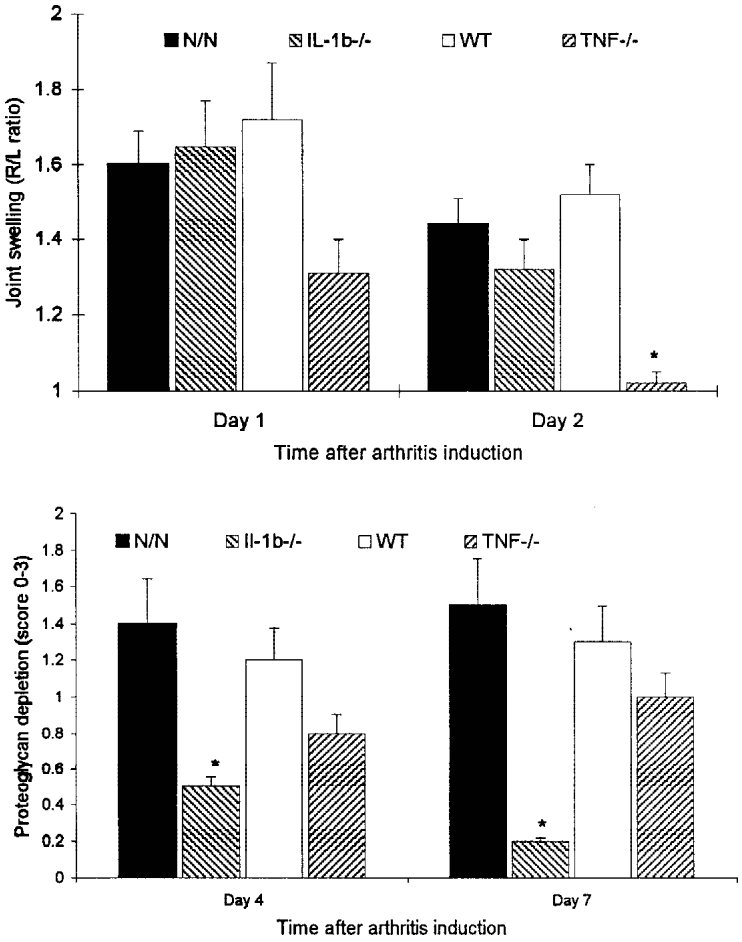

Fig. 4. Joint swelling at days 1 and 2, and proteoglycan loss from the articular cartilage at days 4 and 7 after the induction of SCW arthritis in knockouts and their respective controls (NN for IL-1β -/-, WT for TNFα -/-). Note the TNFα dependency of joint swelling and the IL-1β dependency of proteoglycan loss.

In sharp contrast, joint swelling was not reduced in IL-1β knock-out mice, yet late infiltrate was diminished and cartilage damage was highly reduced. Of interest, chondrocyte PG synthesis inhibition at day 2 was still present in the IL-1β knockouts, thus confirming earlier findings that IL-1α is prominent in early arthritis and can cause PG synthesis inhibition. Early normalisation is seen only when additional anti-IL-1α antibodies are given to IL-1β knock-out mice. Full normalisation of chondrocyte PG synthesis was seen in untreated IL-1β deficient mice at later time points.

A crucial element in the uncoupling of TNFα- and IL-1β-driven events is the independent production of IL-1β. To substantiate this observation, we isolated synovial tissue specimens at 3 and 6 hours after SCW induction in TNFα-deficient mice and measured the IL-1 levels in tissue washouts. A slight reduction in IL-1β levels (20%) was seen at 3 hours, suggesting that some of the initial IL-1β production occurs in a TNFα-dependent fashion. However, at 6 hours no significant difference was seen any more and full amounts of IL-1β (2 ng/knee joint specimen) were produced through a TNFα-independent pathway (24).

The above studies suggest that the potential uncoupling of joint swelling and ongoing cartilage destruction are separate activities of TNFα and IL-1. Although the possibility cannot be excluded at present that the triggering process in human synovial tissue is different, the suggestion that TNFα is the driving force behind IL-1 production, and therefore the key target in RA, must be viewed with caution. Given the pivotal role of IL-1 in amplifying late synovial infiltrate and cartilage destruction, the omission of proper IL-1 blocking might have deleterious consequences for joint damage.

Membrane-bound cytokines

A critical consideration to be made regarding the therapeutic targeting of cytokines relates to the relative roles of the soluble versus the membrane-bound forms of cytokines. Recently it was shown that arthritis could be induced by the transgenic overexpression of transmembrane TNFα (25), thus elegantly demonstrating that soluble TNFα is not needed to result in the full expression of arthritis. First, studies with SCW arthritis in TNFα-deficient mice, knocked in with transmembrane TNFα (mTNF) and kindly provided to us by George Kollias, displayed the full expression of joint swelling, suggesting that mTNFα is fully capable of initiating full-blown joint swelling not only under transgenic overexpression conditions, but also during a normal triggering process with SCW (24). It remains to be seen whether mTNF can mimic all of the aspects attributed to soluble TNF. With regard to cellular infiltration, the total number of cells in the synovial tissue was somewhat reduced compared to those in a littermate control mouse, but was clearly higher compared to the TNFα-/- mice (Fig. 5). Cartilage damage was already high in the TNF-/- mice and, not unexpectedly, was no different in the mTNFα "knock-in" mice.

It is envisaged that local cytokine production within the cartilage and local cytokine receptor expression patterns on chondrocytes are determinants in the process of cartilage destruction in advanced stages of arthritis. However, since chondrocytes are spaced widely apart in an extensive cartilage matrix, it seems unlikely that membrane-bound cytokines are essential elements here, and their role may be confined to processes in densely populated synovial tissue or to sites of pannus tissue eroding the cartilage matrix and allowing for direct synoviocyte/chondrocyte interactions.

Chronic relapsing SCW arthritis

The limited role of TNFα described so far in the tissue pathology in SCW arthritis relates to subacute arthritis. To apply the model more directly to the standard clinical situation, we next evaluated the cytokine interplay in a model of chronic relapsing cell wall-associated

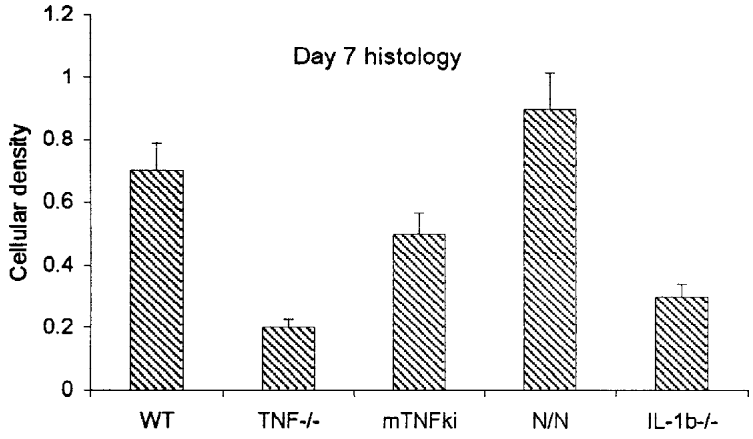

Fig. 5. Cellular infiltrate in the synovial tissue at day 7 of SCW arthritis in the various knockouts. Note the reduction of infiltrate both in the TNFα-/- and the IL-1β-/- mice. The mTNFα knock-in (ki) shows normal infiltration instead.

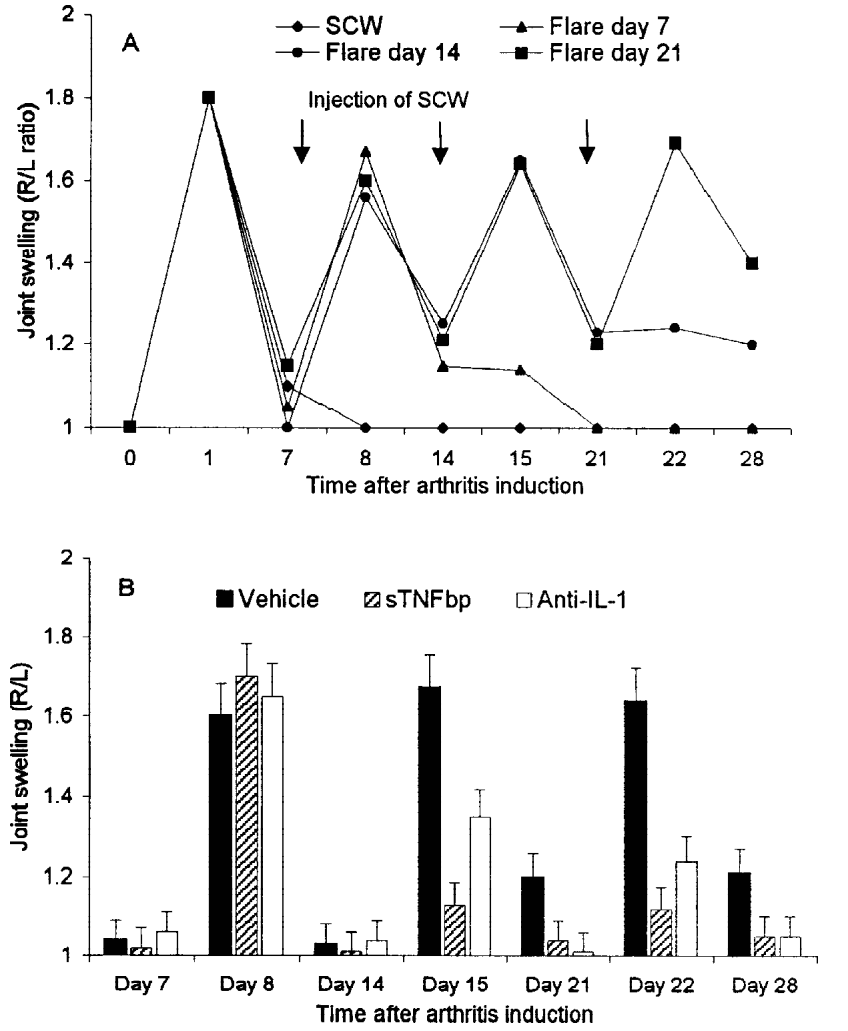

Fig. 6. Chronic relapsing SCW arthritis induced by the repeated injection of SCW fragments into the knee joint. In the upper panel (**A**), it can be seen that chronicity increases after every rechallenge. In the lower panel (**B**), treatment with TNFbp or anti-IL-1(α + β) was given, starting at day 14 with a repeat injection of blockers at day 21.

arthritis. In earlier studies in the mouse, it was shown that a chronic, antigen-induced arthritis can be flared with tiny amounts of antigen when the joint displays local hyperreactivity by retained antigen-specific T cells in the chronic infiltrate (26). Anti-IL-1 treatment appeared effective in such T cell-driven flares and caused a marked reduction of cartilage damage (27). Apart from antigen-specific rechallenge, such a joint is also highly sensitive to flares induced by cytokines such as TNF and IL-1. In fact, it suggests that any non-specific inflammatory insult with the concomitant release of proinflammatory cytokines may flare a chronic arthritis through reactivation of the retained macrophages (28). It also underlines that an arthritis may start with an antigen-specific (auto)immune event, but can be sustained by rather non-specific triggering processes.

In the model of murine SCW arthritis, as well, flares can be induced at sites of smouldering arthritis by rechallenge with small amounts of SCW. An example of repeated flaring with weekly rechallenge intervals is depicted in Figure 6. It shows that acute joint swelling is noted after every rechallenge, but also demonstrates that the remaining smouldering arthritis is brought to a higher level and is becoming more and more severe. Intriguingly, each flare remained TNFα sensitive in terms of joint swelling, as identified by consistent suppression when TNF soluble receptors were given shortly before the flares. However, the chronic cellular infiltrate remained, and advanced cartilage damage was not significantly reduced. In contrast, major protection against ongoing cartilage damage was achieved with anti-IL-1(α + β) antibodies. An increasing effect was also seen on the swelling, probably linked to the sustained effect on the chronic infiltrate (see below). Of interest, Makarov *et al.* showed in a similar model in rats that arthritis could be attenuated when primary synoviocytes were transduced in culture with retroviral IL-1ra and then engrafted into ankle joints prior to the reactivation of arthritis at that site (29).

We also performed repeated flare studies in cytokine knock-out mice. Joint swelling was still significantly reduced after the fourth SCW challenge in TNFα-deficient mice. When the model was analysed in IL-1β-deficient mice, an impressive reduction of cartilage damage was seen. In fact, hardly any erosion was noted, whereas this was abundant in the control and TNFα knock-out mice (Fig. 7). It is of great interest that the chronic synovial infiltrate was also profoundly

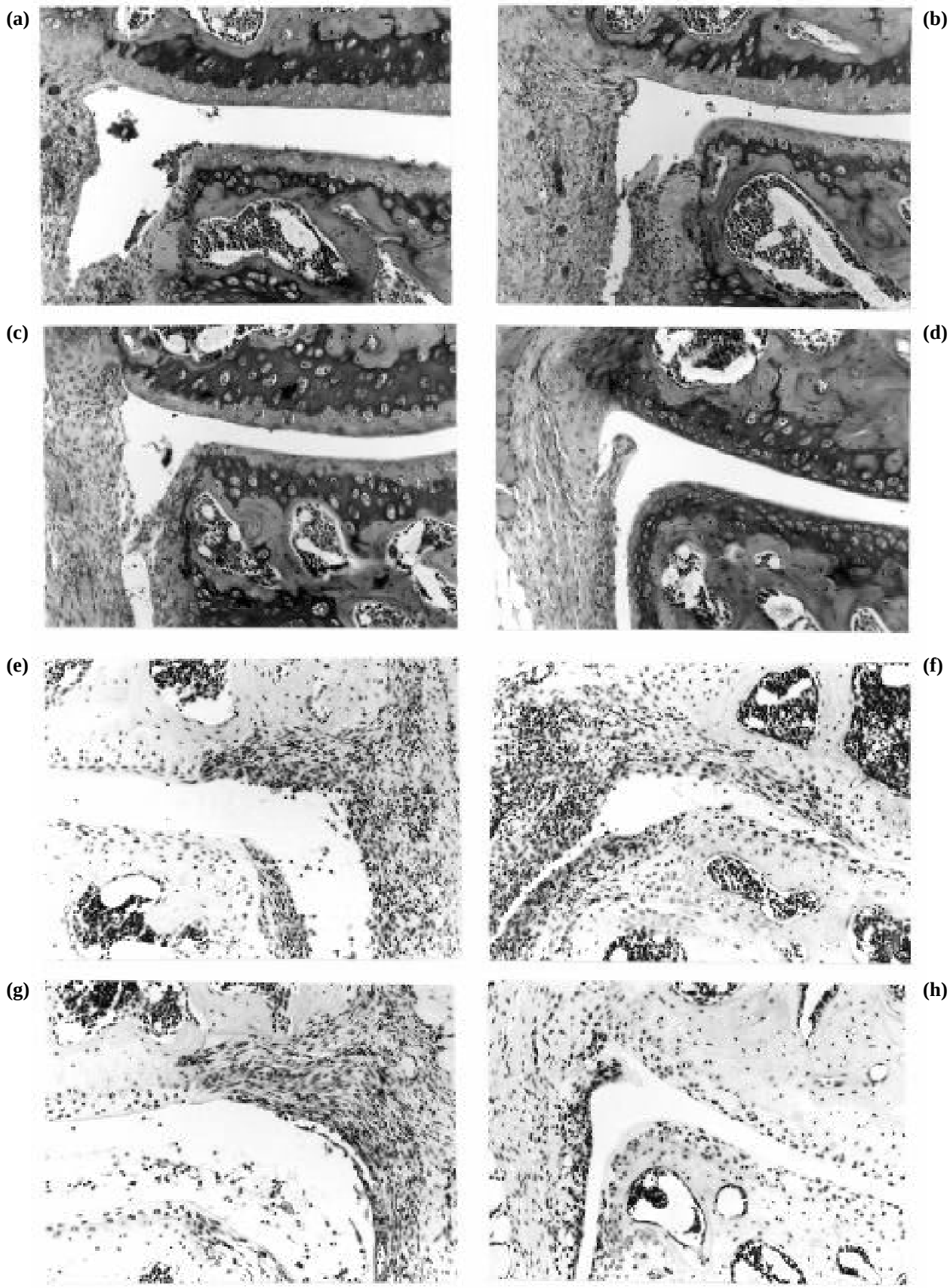

Fig. 7. Histology of the knee at day 28 of the relapsing SCW model (see Fig. 6). Safranin O staining shows loss of proteoglycans from the cartilage surface layer in the controls (**a**, **c**) and the TNFα-/- mice (**b**). In contrast, the IL-1β-/- mice (**d**) show major protection against proteoglycan loss. Hematoxylin staining panels of more superficial sections (**e** and **g**: controls; **f**: TNFα-/-; **h**: IL-1β-/-) show infiltration and ingrowth of synovial tissue at the cartilage margins. This is highly reduced in the IL-1β-/- mice (**h**).

reduced in the IL-1β-deficient mice. This finding suggests that repeated flare reactions render the cellular process in the synovial tissue more and more an IL-1β-dependent phenomenon, and this suppression will contribute to the reduction of subsequent flares. A contributing factor in this suppression might be that the chronicity of inflammation by repeated SCW rechallenge is in part sustained by cartilage molecules released from the damaged tissue and that this contributing factor is high in normal and TNFα-deficient mice, but is almost lacking in IL-1β-deficient mice due to the early arrest of the damaging process. Of note, molecular mimicry has been demonstrated between streptococcal cell wall peptidoglycans and cartilage PG at the T cell level. This may provide yet another contributing element, apart from macrophage activation (30). Further analysis of the cellular process is at present underway to identify the relative roles of macrophage- and T cell-driven elements in the propagation of inflammation and tissue destruction. This includes evaluation of the potential roles of the T cell- and macrophage-associated cytokines IL-12, IL-15, and IL-17 in the chronic stage.

Findings in antigen-induced arthritis

In collagen arthritis and streptococcal cell wall-induced arthritis, IL-1 played a major role not only in cartilage damage but also in the (late) inflammatory process. This suggests that one component of the cartilage protective effect may be viewed as an indirect effect, as a consequence of reduced cellular infiltration. The most impressive proof of a direct, pivotal role of IL-1 in advanced cartilage erosion was obtained from studies in the model of antigen-induced arthritis (AIA). This is a severe, destructive joint inflammation elicited by the direct injection of an antigen into the knee joint of pre-immunized animals. The arthritis is based on antigen retention in the knee joint in the presence of strong T cell-mediated immunity against this retained antigen. Joint swelling was not reduced significantly by anti-IL-1 treatment, nor was the early PG loss from the articular cartilage diminished, thus suggesting overkill by other mediators in these processes. Yet chondrocyte PG synthesis inhibition was fully normalised (31). In addition, net cartilage PG loss measured at day 14 of the arthritis was again profoundly reduced by anti-IL-1 treatment, probably reflecting both the replenishment of lost PG by normalized synthesis and the reduction of further degradation at later stages.

We next evaluated the impact of anti-IL-1 treatment on erosive articular cartilage damage, including an analysis of matrix breakdown neoepitopes and the scoring of chondrocyte death and surface disruption. After the treatment of murine AIA with IL-1ra, early aggrecanase-mediated PG loss was not diminished and this identified an overkill by IL-1 independent enzyme systems. However, the advanced breakdown of PG resulting in loss of the neoepitope NITEGE in the major cartilage PG, aggrecan, and the appearance of another neoepitope, VDIPEN, was fully prevented (32, 33). This absence of VDIPEN expression correlated with the lack of erosive changes and chondrocyte death in the cartilage after 1 - 2 weeks. Studies in stromelysin-deficient mice identified a crucial role of stromelysin in advanced PG breakdown, collagenase activation, and collagen damage (34). Intriguingly, IL-1 is not necessarily a crucial mediator in the early PG loss mediated by aggrecanase, but is a pivotal factor in stromelysin activation and erosive cartilage destruction.

Clinical relevance

The above studies using various models imply that IL-1 is always a major destructive cytokine, but that it can be a major factor in chronic synovial inflammation as well. This argues that IL-1 would be a pivotal therapeutic target. To date, this broad profile, with a role in inflammation as well, is not supported by observations of IL-1-directed therapy in RA patients, since suggestive proof has been obtained only for a role in erosions and not in inflammation (4). Whether this argues that the inflammatory elements of the RA process are different from the aspects of the various models remains to be identified. Current clinical studies are hampered by the absence of high-quality IL-1-neutralising antibodies and by the poor pharmacokinetics of IL-1ra, requiring continued high dosing. At present, it cannot be excluded that the studies to date are underdosing and therefore are not addressing IL-1-dependent processes. Unfortunately, clinical studies with scavenging soluble IL-1 receptors have been performed thus far with the type I receptor, which has poor affinity for IL-1β and high affinity for IL-1ra. Studies with the decoy type II receptor are still awaited.

The alternative approach of direct IL-1 neutralization would be to block an upstream mediator, which generates IL-1 production. This is the goal of anti-TNFα-directed therapy in RA patients. The basis for this approach is the suggestion that TNFα is the driving force of IL-1 in the synovial tissue in human RA (35), a hypothesis based on early observations with neutralising anti-TNF antibodies and cytokine production in RA synovial tissue *in vitro* (35). However, additional support from follow-up studies by other groups is unfortunately lacking so far.

Samples from the early stages of RA are now becoming available through arthroscopic biopsies and blind small-needle biopsies. TNF and IL-1 are not always found, and the mRNA expression of these cytokines does occur uncoupled (36). Our personal impression from biopsies is that a large variation exists in TNFα and IL-1 immunolocalization patterns in specimens from different RA patients. Moreover, our preliminary observations in biopsies from RA patients treated with fully humanized anti-TNFα antibodies could not identify a significant reduction of IL-1 in the synovial cells. On the other hand, it is clear that systemic anti-TNFα treatment does reduce IL-1 mRNA in the circulating leucocytes in most patients, while it provides impressive, symptomatic relief. Further studies are awaited that will identify the components of the synovial reaction and the specific cytokine interplay in individual RA patients. It is to be expected that we are dealing with a heterogeneous process in various patients, and that the tailor-made therapy will not necessarily be identical for all patients. At present a double-hit approach, directed at TNFα and IL-1β, appears warranted.

Final remarks regarding combination therapy

Based on both clinical and experimental data, TNFα is a logical target to provide relief of the symptoms of pain and swelling. However, since marked uncoupling may occur between joint swelling and the erosion of cartilage, combination therapy with an IL-1 inhibitor represents a logical extension, and experimental data suggest that IL-1β is the more dominant target of the IL-1 isoforms. Intriguingly, since part of the PG loss in articular cartilage may occur in an IL-1-independent fashion, the addition of particular enzyme inhibitors might be considered. It is still unclear which portions of the destructive enzymes are produced by synovial macrophages, fibroblasts, or articular chondrocytes, and how much of their production is regulated by the cytokines TNFα and IL-1β and how much occurs independently. In that sense, combination therapy which reduces the synovial cell mass or cellular activation may provide relief in addition to monotherapy.

Apart from the inhibition of proinflammatory and destructive cytokines, the addition of regulatory cytokines can offer an alternative approach. In experimental arthritis models, it appeared that combination treatment with IL-4 and IL-10 was far superior to IL-4 or IL-10 alone, particularly in reducing cartilage damage (37). Further studies revealed that IL-10 was a potent TNFα inhibitor, but consistent suppression of IL-1 was only noted after combination treatment to suppress IL-4/IL-10. IL-4 inhibits cytokine production in the synovial tissue, but also exerts direct protective effects on chondrocytes through the upregulation of cytokine inhibitors. Intriguingly, local gene therapy with IL-4 did not reduce local inflammation, but provided impressive protection against cartilage and bone erosion (38). Protection against bone erosion appeared to be linked to reduced levels of the novel bone resorptive factor osteoprotegerin-ligand (39) and to the absence of osteoclast activation. Preliminary studies in experimental murine arthritis models with the combination of IL-4 and low-dose steroid suggested that the cartilage and bone protective capacity of IL-4 could be retained, whereas a low dose of steroid was sufficient to provide the additional relief of inflammatory symptoms. Whether this combination can be safely applied to RA patients remains to be seen.

References

1. FIRESTEIN GS, ZVAIFLER NJ: How important are T cells in chronic rheumatoid synovitis. *Arthritis Rheum* 1990; 33: 768-73.
2. FELDMANN M, BRENNAN F, MAINI RN: Role of cytokines in rheumatoid arthritis. *Annu Rev Immunol* 1996; 14: 397-440.
3. AREND WP, DAYER JM: Inhibition of the production and effects of IL-1 and TNFα in RA. *Arthritis Rheum* 1995; 38: 151-60.
4. BRESNIHAN B, ALVARO-GARCIA JM, COBBY M *et al.*: Treatment of rheumatoid arthritis with recombinant human IL-1 receptor antagonist. *Arthritis Rheum* 1998; 41: 2196-204.
5. VAN DE LOO AAJ, VAN DEN BERG WB: Effects of murine recombinant IL-1 on synovial joints in mice: Measurement of patellar cartilage metabolism and joint inflammation. *Ann Rheum Dis* 1990; 49: 238-45.
6. HENDERSON B, PETTIPHER ER: Arthritogenic actions of recombinant IL-1 and TNFα in the rabbit; evidence for synergistic interactions between cytokines *in vivo. Clin Exp Immunol* 1989; 75: 306-10.
7. GHIVIZZANI SC, KANG R, GEORGESCU HI *et al.*: Constitutive intra-articular expression of human IL-1β following gene transfer to rabbit synovium produces all major pathologies of human rheumatoid arthritis. *J Immunol* 1997; 159: 3604-12.
8. NIKI Y, YADMADA H, KIKUCHI T, TAKAISHI H, FUJIKAWA K, TADA N: Membrane associated IL-1 contributes to chronic synovitis in human IL-1ra transgenic mice. *Arthritis Rheum* 1998; 41: S212.
9. PROBERT L, PLOWS D, KONTOGEORGOS G, KOLLIAS G: The type I IL-1 receptor acts in series with TNFα to induce arthritis in TNFα transgenic mice. *Eur J Immunol* 1995; 25: 1794-7.
10. JOOSTEN LAB, HELSEN MMA, VAN DEN BERG WB: Accelerated onset of collagen-induced arthritis by remote inflammation. *Clin Exp Immunol* 1994; 97: 204-11.
11. JOOSTEN LAB, LUBBERTS E, HELSEN MMA, VAN DEN BERG WB: Dual role of IL-12 in early and late stages of murine collagen type II arthritis. *J Immunol* 1997; 159: 4094-102.
12. WILLIAMS RO, FELDMANN M, MAINI RN: Anti-TNF ameliorates joint disease in murine collagen induced arthritis. *Proc Natl Acad Sci USA* 1992; 89: 9784-8.
13. WOOLEY PH, DUTCHER J, WIDMER MB, GILLIS S: Influence of a recombinant human soluble TNF receptor Fc fusion protein on type II collagen induced arthritis in mice. *J Immunol* 1993; 151: 6602-7.
14. VAN DEN BERG WB, JOOSTEN LAB, HELSEN MMA, VAN DE LOO AAJ: Amelioration of established murine collagen induced arthritis with anti-IL-1 treatment. *Clin Exp Immunol* 1994; 95: 237-43.
15. JOOSTEN LAB, HELSEN MMA, VAN DE LOO FAJ, VAN DEN BERG WB: Anticytokine treatment of established type II collagen-induced arthritis in DBA/1 mice: A comparative study using anti-TNFα, anti-IL-1α/β, and IL-1ra. *Arthritis Rheum* 1996; 39: 797-809.
16. BAKKER AC, JOOSTEN LAB, ARNTZ OJ *et al.*: Prevention of murine collagen-induced arthritis in the knee and ipsilateral paw by local expression of human IL-1ra protein in the knee. *Arthritis Rheum* 1997; 40: 893-900.
17. KU G, FAUST T, LAUFFER LL, LIVINGSTON DJ, HARDING MW: IL-1β converting enzyme inhibition blocks progression of type II collagen induced arthritis in mice. *Cytokine* 1996; 8: 377-86.
18. PASPARAKIS M, ALEXOPOULOU L, EPISKOPOU V, KOLLIAS G: Immune and inflammatory responses in TNFα-deficient mice: A critical requirement for TNFα in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *J Exp Med* 1996; 184: 1397-411.
19. MORI L, ISELIN S, DE LIBERO G, LESSLAUER W: Attenuation of collagen-induced arthritis in 55-kDa TNF receptor type 1 (TNFR1)-IgG1-treated and TNFR1-deficient mice. *J Immunol* 1996; 157: 3178-82.
20. JOOSTEN LAB, HELSEN MMA, SAXNE T, VAN DE LOO FAJ, HEINEGARD D, VAN DEN BERG WB: IL-1α,β blockade prevents cartilage and bone destruction in murine type II collagen-induced arthritis, whereas TNFα blockade only ameliorates joint inflammation. *J Immunol* (in press).
21. LUBBERTS E, JOOSTEN LAB, HELSEN MMA, VAN DEN BERG WB: Regulatory role of IL-10 in joint inflammation and cartilage destruction in murine streptococcal cell wall (SCW) arthritis. More therapeutic benefit with IL-4/IL-10 combination therapy than with IL-10 treatment alone. *Cytokine* 1998; 10: 361-9.
22. KUIPER S, JOOSTEN LAB, BENDELE AM *et al.*: Different roles of TNFα and IL-1 in murine streptococcal cell wall arthritis. *Cytokine* 1998; 10: 690-702.
23. ZHENG H, FLETCHER D, KOZAK W *et al.*: Resistance to fever induction and impaired acute-phase response in IL-1β-deficient mice. *Immunity* 1995; 3: 9-19.
24. VAN DEN BERG WB, JOOSTEN LAB, KOLLIAS G, VAN DE LOO FAJ, HELSEN MMA: TNFα independent role of IL-1β in cartilage destruction in SCW arthritis: Evidence from TNFα and IL-1β deficient mice. Submitted.
25. GEORGOPOULOS S, PLOWS D, KOLLIAS G: Transmembrane TNF is sufficient to induce localized tissue toxity and chronic inflammatory arthritis in transgenic mice. *J Inflammation* 1996; 46: 86-97.
26. LENS JW, VAN DEN BERG WB, VAN DE PUTTE LBA, ZWARTS WA: Flare-up of antigen induced arthritis in mice after challenge with intravenous antigen: Kinetic of antigen in the circulation and localization of antigen in the arthritic and noninflamed joint. *Arthritis Rheum* 1986; 29: 665-74.
27. VAN DE LOO AAJ, ARNTZ OJ, BAKKER AC, VAN LENT PLEM, JACOBS MJM, VAN DEN BERG WB: Role of interleukin-1 in antigen-induced exacerbations of murine arthritis. *Am*

J Pathol 1995; 146: 239-49.

28. 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.
29. MAKAROV SS, OLSEN JC, JOHNSTON WN *et al.*: Suppression of experimental arthritis by gene transfer of IL-1ra cDNA. *Proc Natl Acad Sci USA* 1996; 93: 402-6.
30. VAN DEN BERG WB, VAN DEN BROEK MF, VAN DE PUTTE LBA, VAN BRUGGEN MCJ, VAN LENT PLEM: Experimental arthritis: Importance of T cells and antigen mimicry in chronicity and treatment. *In:* KRESINA TF (Ed.): *Monoclonal Antibodies, Cytokines, and Arthritis*. Dekker, 1991; 237-52.
31. VAN DE LOO AAJ, JOOSTEN LAB, VAN LENT PLEM, ARNTZ OJ, VAN DEN BERG WB: Role of interleukin-1, tumor necrosis factor α and interleukin-6 in cartilage proteoglycan metabolism and destruction. Effect of *in situ* cytokine blocking in murine antigen- and zymosan-induced arthritis. *Arthritis Rheum*, 1995; 38: 164-72.
32. VAN MEURS JBJ, VAN LENT PLEM, HOLTHUYSEN AEM, SINGER II, BAYNE EK, VAN DEN BERG WB: Kinetics of aggrecanase and metalloproteinase induced neoepitopes in various stages of cartilage destruction in murine arthritis. *Arthritis Rheum* 1999; 42: 1128-39.
33. VAN MEURS JBJ, VAN LENT PLEM, SINGER II, BAYNE EK, VAN DE LOO FAJ, VAN DEN BERG WB: IL-1ra prevents expression of the metalloproteinase-generated neoepitope VDIPEN in antigen-induced arthritis. *Arthritis Rheum* 1998; 41: 647-56.
34. VAN MEURS JBJ, VAN LENT PLEM, HOLTHUYSEN AEM *et al.*: Cleavage of aggrecan at Asn341-Phe342 site coincides with the initiation of collagen damage in murine antigen-induced arthritis: A pivotal role for stromelysin-1 in MMP activity. *Arthritis Rheum* (in press).
35. BRENNAN FM, CHANTRY D, JACKSON A, MAINI RN, FELDMANN M: Inhibitory effect of TNFα antibodies on synovial cell IL-1 production in rheumatoid arthritis. *Lancet* 1989; ii: 244-7.
36. DELEURAN B: Cytokines in RA; localization in arthritic joint tissue and regulation *in vitro*. *Scand J Rheumatol* 1996; 25 (Suppl.104): 1-38.
37. JOOSTEN LAB, LUBBERTS E, DUREZ P *et al.*: Role of IL-4 and IL-10 in murine collagen-induced arthritis: Protective effect of IL-4 and IL-10 treatment on cartilage destruction. *Arthritis Rheum* 1997; 40: 249-60.
38. LUBBERTS E, JOOSTEN LAB, VAN DEN BERSSELAAR L *et al.*: Adenoviral vector-mediated overexpression of IL-4 in the knee joint of mice with collagen-induced arthritis prevents cartilage destruction. *J Immunol*, in press.
39. KONG YY, YOSHIDA H, SAROSI I, *et al.*: OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* 1999; 397: 315-23.