Arguments for interleukin 1 as a target in chronic arthritis

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Abstract

Tumour necrosis factor (TNF) and interleukin 1 (IL1) are considered as master cytokines in chronic, destructive arthritis. Therapeutic approaches in rheumatic arthritis (RA) patients so far mainly focused on TNF. Although TNF is a major inflammatory mediator in RA and a potent inducer of IL1, anti-TNF treatment is not effective in all patients, nor does it fully control the arthritic process in affected joints of good responders. Analysis of cytokine patterns in early synovial biopsy specimens of RA patients reveals prominent TNF staining in 50% of the patients, whereas IL1b staining was evident in 100%. This argues that TNF independent IL1 production occurs in some of the patients. Studies in a range of experimental arthritis models in mice make it clear that TNF is involved in early joint swelling. However, TNF alone is not arthritogenic nor destructive and exerts its arthritogenic potential through IL1 induction. Intriguingly, TNF independent IL1 production is found in many models. Its relevance is further underlined by the greater efficacy of anti-IL1 treatment as compared with anti-TNF treatment and the total lack of chronic, erosive arthritis in IL1b deficient mice. IL1b is not necessarily involved in early joint swelling, but is a crucial mediator in chronic arthritis and cartilage erosion in all models studied so far. This makes IL1b an attractive target in chronic, destructive arthritis.

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Arthritogenic potency of tumour necrosis factor and interleukin 1

It is now generally accepted that arthritis can be induced in mice by tumour necrosis factor (TNF) as well as interleukin 1 (IL1). This is first demonstrated by local injection of recombinant cytokines in the knee joint, and substantiated by studies in transgenic mice and induction of arthritis by local cytokine overexpression in joint tissues with viral vectors. Intriguingly, IL1 is much more potent as compared with TNF, to induce cartilage destruction in vivo. Tiny amounts of IL1 are already sufficient to cause chondrocyte proteoglycan

synthesis inhibition, whereas roughly a 100-fold to 1000-fold higher dose of TNF is needed to obtain the same effect.¹ Of importance, synergy between IL1 and TNF has been seen. Apart from potency differences, it is clear that it is hard to measure significant TNF levels in inflamed synovial tissue or synovial fluid and the levels are certainly not higher as compared with IL1. It should be noted that most effects might be related to membrane bound forms of cytokines, which are hard to measure. On the other hand, impact on articular cartilage from synovium derived mediators probably needs traffic of soluble forms.

A further argument for the limited, direct role of TNF in arthritis emerged from elegant studies in TNF transgenic mice. Joint inflammation was completely arrested when these mice were treated with anti-IL1 receptor antibodies.² This argues that the pathology runs through the induction of IL1, which is the real arthritogenic trigger, either alone or in synergy with TNF. TNF levels were still high after anti-IL1R treatment, which implies that TNF alone is hardly arthritogenic.

Final support for the crucial role of IL1 emerged from the recent demonstration of spontaneous arthritis in Balb/c mice, deficient in IL1 receptor antagonist (IL1Ra) (table 1).³ Earlier work already identified higher susceptibility of IL1Ra deficient DBA mice for collagen induced arthritis.⁴ The occurrence of spontaneous arthritis, when the IL1Ra deficiency was backcrossed to a Balb/c genetic background, illustrates the continued arthritogenic pressure of environmental IL1, which is normally controlled by the endogenous IL1Ra.

TNF independent IL1 production in models, when?

The above reasoning does not exclude TNF as a major therapeutic target. There is an old claim that spontaneous IL1 production in RA synovial tissue is TNF dependent.⁵ However, these findings have not been confirmed so far. Moreover, in experimental model situations there is now ample evidence of direct IL1 generation.

To further our understanding of relative TNF dependency of IL1 production under various arthritogenic conditions, we compared the efficacy of TNF and IL1 blocking in a range of experimental arthritis models, including immune and non-immune triggering. Moreover, similar analysis of models was done in TNF and IL1b deficient mice.

Major findings are summarised in table 2 and can be found in several studies.⁶⁻²⁰ IL1 is not necessarily a dominant cytokine in the acute, inflammatory stages of most arthritis

Table 1 Arguments for a dominant role of IL1 in destructive arthritis

Higher arthritogenic potency of IL1 compared with TNF
TNF induced arthritis can be fully blocked with IL1R antibodies
TNF independent IL1 production occurs in many arthritis models
Greater net efficacy of anti-IL1 as compared with anti-TNF treatment in many models
Erosive arthritis can still be induced in TNF deficient mice
No erosive experimental arthritis in IL1b deficient mice
Spontaneous destructive arthritis occurs in IL1Ra deficient mice

Table 2 Cytokine dependence of various murine arthritis models

Arthritis model	Details/principal target or trigger		Early inflammation cytokine involvement		Erosive arthritis cytokine involvement	
	References		TNF	IL1	TNF	IL1
SCW -A	6,7	Macroph.	++	-	-	++
SCW flares	8-10	T cells	+	+	-	++
AIA	11,12	T cells/IC	±	±	-	++
AIA flare	13	T cells	±	+	-	++
CIA	14-19	T cells/IC	+	++	++	++
ICA	20	IC	-	++	-	++

Cytokine involvement, based on experiments with neutralising antibodies, started at the onset of arthritis. It does not reflect potential involvement during preimmunisation (CIA and AIA).

SCW = streptococcal cell wall fragments.

SCW-A flares reflect the situation after three consecutive SCW flares with seven days interval.

AIA = antigen induced arthritis.

AIA flare is induced by antigen rechallenge at day 21.

CIA = collagen induced arthritis.

ICA = passive immune complex (IC) arthritis.

*Efficacy of anti-TNF treatment only when started at onset of first signs of arthritis.

models, but plays a crucial part in propagation of joint inflammation and concomitant cartilage and bone erosion in all models. The fact that the chronic, destructive stage is IL1 and not TNF dependent indirectly proves that TNF independent IL1 production occurs under all experimental model conditions listed.

Most abundant TNF dependence of acute inflammation is found when arthritis is induced with a phagocytic trigger such as streptococcal cell walls or Zymosan (yeast particle). However, erosions still develop in these models after treatment with anti-TNF antibodies and this observation has been strengthened by the high degree of erosions when such models were induced in TNF deficient mice. Not surprisingly, IL1 levels were still high under these conditions, identifying considerable TNF independent IL1 triggering (fig 1). When repeat injections with SCW fragments were given, the inflammation became partly IL1 dependent, erosions still developed in TNF deficient mice and were fully absent in IL1b deficient mice. It is expected that repeat SCW injection will generate specific T cell immunity and that the repeated flare model becomes more T cell dependent. Studies are in progress to elucidate the relative role of TNF and lymphotoxin, using double knockout mice. First data suggest that synovial cell density in chronic SCW arthritis is even more pronounced in TNF deficient as compared with normal mice, suggesting a homeostatic role of TNF in control of synovial cell survival. This implies that full TNF blockade should be avoided in therapeutic approaches. Arthritis appeared clearly reduced in lymphotoxin deficient mice.

Dominant IL1 dependence of immune complex arthritis

A remarkable finding was the strong IL1 dependence of the inflammatory response induced with plain immune complexes.²⁰ The dominant role of IL1 in both inflammation and destruction of collagen induced arthritis suggests that immune complex mediated events are a crucial element in this model. Apparently, T cells are mainly important in early stages of collagen arthritis, to support sufficient collagen type II autoantibody production. Recently, a novel autoimmune arthritis model was gener-

Figure 1 IL1b levels in tissue washouts six hours after injection of SCW fragments into the knee joint of mice. The first set depicts IL1 levels in control and anti-TNFα treated mice (see also van den Berg⁶). The second set depicts values in control and TNF-/- mice. Although some reduction is consistently noted in TNF-/- mice, it does not reach statistical significance, implying that most of the IL1 is produced in a TNF independent fashion.

ated in KRN mice by transgenic overexpression of a T cell receptor, directed against MHC molecules. This transgenic condition resulted in skewed control of tolerance and was characterised by significant autoantibody formation. The crucial observation was that the model was transferrable with purified autoantibodies.²¹ Of high interest, this model appeared not TNF but IL1 dependent (personal observations).

Cartilage erosion

Destruction of articular cartilage is caused by the combination of inhibited synthetic activity of the articular chondrocytes and enzymatic breakdown of the matrix. The latter can be elicited by enzymes released from chondrocytes and/or the inflamed synovial tissue, in particular at sites of so called pannus overgrowth of the cartilage. Early changes are characterised by loss of proteoglycans, which in principle is a reversible process. A major step in erosive tissue loss is the destruction of collagen bundles. Intriguingly, IL1 is very potent in inducing suppression of matrix synthesis by the chondrocytes. It also induces release of active aggrecanase, which is the dominant enzyme responsible for proteoglycan loss. In contrast, IL1 induces the release of latent forms of metalloproteinases, including stromelysin (MMP-3) and collagenase (MMP-13). The latter is crucial in collagen breakdown and stromelysin seems pivotal in collagenase activation.²²⁻²⁴ IL1 alone gives limited cartilage erosions, linked to moderate MMP autoactivation. In the presence of immune complexes in the joint, IL1 induced, latent MMPs become broadly activated and cause major tissue erosion.²⁵ Fc receptor binding on leucocytes and/or chondrocytes and release of activating mediators are a crucial element in this immune complex mediated activation step. Cartilage erosion is absent in antigen induced arthritis elicited in Fc receptor deficient mice despite florid joint inflammation.²⁶ In addition, IL1Ra treatment prevents erosions and MMP activity in this model, with limited suppression of acute joint inflammation.²³ These findings identify IL1 as a pivotal initiating step in erosive processes and underline the role of immune complexes in exaggeration of destruction. Rheumatoid factor positivity is correlated to

Figure 2 Simplified version of cytokine involvement in osteoclast activation.

more severe and destructive forms of RA, which may fit with the above concept.

Bone erosion

Apart from cartilage damage, chronic arthritis is characterised by erosions of the underlying bone. The recently identified osteoprotegerin-ligand (OPG-L) seems a crucial mediator in this process,²⁷ as bone loss was absent in OPG-L deficient mice. OPG-L is the pivotal mediator of osteoclast differentiation and activation. Its production can be stimulated by a range of cytokines. IL1 is the most potent activator and is also a dominant factor in osteoclast differentiation (fig 2). TNF and IL17 are less potent, but display considerable synergy. IL17 is a T cell derived cytokine, sharing many properties with IL1. It remains to be identified whether OPG-L is a good target for prevention of bone erosion, or whether it is more safe to target the inducing and modulating cytokines. An obvious side effect of direct OPG-L blocking is the unwanted interference with normal bone turnover.

OPG-L activity is controlled by a soluble receptor (OPG), and the balance of OPG-L/OPG is crucial in this. Recently, we found that IL4 can protect against cartilage and bone erosion in collagen arthritis. IL4 improves the OPG-L/OPG balance and suppresses IL17, but also upregulates IL1Ra and markedly reduces IL1.^{28,29} It is still unclear whether the protective effect of IL4 is mainly linked to IL1 suppression, but it is evident that IL1 is a major player in bone erosion in arthritis.

The clinical situation, anti-cytokine treatments

It has long been recognised that treatment of RA patients with IL1Ra is marginally protective in terms of joint swelling, but is clearly protective against erosive changes.³⁰ This pattern is most compatible with the findings of IL1 blocking in murine antigen induced arthritis.¹¹ IL1Ra has a weak pharmacokinetic profile and it is still unclear from the clinical studies whether the limited effect on joint inflammation is akin to the RA process or related to suboptimal blocking of IL1. The experience from animal model studies with IL1Ra teaches that continued dosing with Alzet minipumps is the proper way to fully control IL1.

Remarkably, the recent evaluation of joint erosions after anti-TNF treatment of RA patients also provided evidence for a protective

Figure 3 Potential pathways of TNF overproduction. Note that general T cell/macrophage triggering, as studied in arthritis models, gives rise to both TNF and IL1, with considerable TNF independent IL1 production, and extreme skewing to IL1 when immune complexes are used as stimulus.

Figure 4 Amplifying elements in erosive processes. Immune complexes generate high levels of IL1 and through Fc interaction also provide additional mediators to activate pro-MMPs (metalloproteinases). T cells may be involved in enhanced bone erosion through TNF, IL17 and direct OPG-L production. T cells come close to the bone at erosion sites. IL17 also promotes cartilage erosion, a role of OPG-L in this remains to be seen.

effect. This may fit with the hypothesis that TNF overproduction in RA synovial tissue is mainly caused by deranged behaviour of synovial cells, displaying too much TNF generation. If this is the case, TNF will drive IL1 production and TNF blockade will be sufficient to control this pathway (fig 3). However, it remains to be seen whether these observations can be accepted as proof of concept. Anti-TNF antibodies used in the clinical studies display cytotoxic effects. This may imply that one mechanism of the anti-TNF effect is linked to binding to TNF bearing cells and subsequent killing of these cells, potentially including TNF/IL1 producing cells or neighbouring cells. In addition, the TNF soluble receptor used in some of the anti-TNF trials binds not only to TNF but also scavenges lymphotoxin. The latter may have an impact on T cell driven pathways. A final comment to be made here is that analysis of anti-erosive efficacy in clinical trials is mainly based on bone erosions. Focal damage of cartilage is more difficult to score on radiographs. It remains to be seen whether the relative dominance of TNF/IL1 involvement and amplifying elements (fig 4) are similar or different in cartilage and bone destruction in RA.

Heterogenous synovial cytokine patterns in RA patients

Early biopsy specimens taken from knee joints of RA patients with limited disease duration identified a variable pattern of cytokines.^{31,32}

Table 3 Immunostaining of cytokines in early synovial biopsy specimens of RA patients

Cytokine	RA patients	Positive staining (%)
TNF	n=28	50
IL1b	n=28	100
IL17	n=28	70

Five blind synovial needle biopsy specimens per RA patient were taken and three sections of at least three suitable (sufficient cellularity) biopsy specimens were evaluated. Staining is considered negative when mean values were not significantly different from control staining with irrelevant antibodies.

Although TNF was abundantly present in some RA patients, TNF was undetectable in half of the patients. In contrast, IL1b was found in all RA synovial biopsy specimens studied so far (table 3). Remarkably, distinct IL17 staining was also clearly present in 70% of the RA patients, arguing a reconsideration of T cell involvement.

Repeat biopsy specimens taken two weeks after the start of successful systemic anti-TNF treatment did not identify significant suppression of IL1 staining in the synovial tissue in our hands. This may be interpreted as suggestive evidence of a lack of a TNF-IL1 cascade in many patients, but it may also suggest that the beneficial clinical effect is mainly attributable to systemic cytokine blocking,³² without a rapid, consistent impact on local events. Studies are in progress to evaluate repeat biopsy specimens after more prolonged anti-TNF treatment.

The heterogeneity in cytokine patterns argues for different disease pathways in various RA patients or depicts various stages of the disease. It is clear from the experimental arthritis model studies that many pathogenic pathways cause TNF independent IL1 production, with a particular skewing to IL1 dominance in immune complex mediated events (fig 4).²⁰ It seems obvious that future treatment will consist of combination treatment, at least touching both TNF and IL1. It is tempting to speculate that tailor-made cytokine directed treatment will be applicable in the near future, based on individual cytokine patterns.

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