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**ABSTRACT**

TNF and IL-1 are master cytokines in chronic destructive arthritis. Therapeutic approaches have so far focused mainly on TNF, which is a major inflammatory mediator in RA and a potent inducer of IL-1. Anti-TNF treatment shows great efficacy in RA patients. However, it is not effective in all patients, nor does it fully control the arthritic process in affected joints of good responders. Although TNF is an early mediator and often crucial in onset of experimental arthritis, studies in rodent models revealed that TNF independent IL-1 production does occur in many arthritic situations. These include direct, innate macrophage activation by phlogistic stimuli, but also immune driven conditions of immune complex and T cell mediated arthritis. If elements of the models apply to the arthritic process in RA patients, it is necessary to block IL-1 in addition to TNF.

**Introduction**

Studies in experimental models of arthritis can be used to identify the relative role of TNF and IL-1 in defined arthritic conditions (1-3). In the subsequent sections a set of animal model situations will be discussed, ranging from TNF overexpression to bacterial activation of synovial cells and antibody and T cell mediated triggering of arthritis. Potential relevance for optimal treatment of RA is discussed.

**Arthritogenic potency of TNF is dependent on IL-1**

The belief in TNF as a major mediator of arthritis emerged from the elegant demonstration of chronic, erosive arthritis in transgenic mice, displaying general TNF overexpression (4). Intriguingly, this arthritis could not only be blocked with TNF scavengers, but also with antibodies to the IL-1 receptor (5). This identifies that TNF sets the process in motion. However, the pathology runs through induction of IL-1, which is the real arthritogenic mediator, either alone or in synergy with TNF. Of note, TNF levels were still high after treatment with antibodies against IL-1 receptor, which implies that TNF alone is hardly arthritogenic. This fits with the poor capacity of recombinant TNF to induce significant arthritis after direct injection in knee joints of rodents, in marked contrast to the strong potency of IL-1 (6). Synergy between TNF and IL-1 has been demonstrated (7).

**Relative role of TNF and IL-1 in various stages of collagen arthritis**

Apart from differences in arthritogenic potencies the relative role of TNF and IL-1 is determined by the occurrence of these cytokines at various stages of the arthritic process. It was a major breakthrough to note that collagen type II arthritis, the classic autoimmune model of RA in rodents, which is a mixture of immune complex and T cell driven arthritis, could be suppressed with anti-TNF antibodies or TNF soluble receptors (8, 9). It is now generally accepted that TNF is mainly involved in onset of collagen arthritis, with efficient blockade when anti-TNF treatment was started before or shortly after onset of arthritis, but showing poor effects in advanced disease (Fig. 1).

In contrast, anti-IL-1 treatment was highly efficient both in early and advanced stages and also arrested progressive joint destruction (9). Recent studies in TNF receptor knockout mice, as well as TNF and IL-1 deficient mice confirmed these findings (10,11). Incidence of arthritis was reduced in TNFR or TNF -/- mice, but once first signs of arthritis developed in some of the mice, progression to full blown, destructive arthritis was a common feature. It identifies that TNF is a susceptibility factor, facilitating onset of arthritis, but progression of arthritis can occur in a TNF independent fashion. The fact that full
blown arthritis was never seen in IL-1 deficient mice substantiates that IL-1 is pivotal in progression (12). Further evaluation of anti-collagen type II immunity in IL-1 deficient mice made it clear that antibody levels were undisturbed but anti-collagen type II T cell reactivity was markedly suppressed. This fits with the concept that collagen arthritis starts mainly as an immune complex arthritis, with subsequent maturation of anti-CII T cell reactivity and propagation of arthritis predominantly through T cells, in an apparent TNF independent but IL-1 dependent fashion.

Immune complex arthritis

Observations of cytokine dependence of various models of plain immune complex arthritis are roughly in line with the findings in collagen arthritis. In the absence of IL-1 none of the immune complex models showed significant inflammation or tissue destruction (13,14), herein identifying a consistent, pivotal role of IL-1.

With respect to TNF dependence, the story seems more complicated. For instance, passive immune complex arthritis induced by local injection in the joint of poly-L-lysozyme, followed by systemic injection of anti-lysozyme antibodies, showed independence of TNF (13). Antibodies to TNF did not ameliorate the arthritic process and the model runs undisturbed in TNF deficient mice. In marked contrast, incidence of autoimmune arthritis, induced by transfer of antibodies directed against the abundant self-antigen GPI (glucose phosphate isomerase) was markedly reduced in TNF deficient mice, although it was confusing to note that TNF receptor knockout mice were susceptible. The few TNF deficient animals which did develop arthritis showed a destructive phenotype, with propagation in a TNF independent fashion (14). These contrasting findings in the lysozyme and GPI model are still consistent with a facilitating role of TNF in onset. Facilitation seems a crucial initiating element in passive GPI arthritis, where antibodies have to settle to the joint. In the lysozyme model, a cationic antigen is planted in the joint and the cationic nature of the antigen provokes sufficient nonspecific inflammation on its own to enhance vascular leakage, influx of antibodies and leucocytes, with subsequent local immune complex formation and further infiltration of cells.

In addition to the nature of the (auto)antigen, differences between relative TNF dependence of IC arthritis models might also be linked to variations in antibody subtypes and pathways of complement activation. The GPI arthritis is mainly caused by IgG1 antibodies, whereas the dominant antibodies in autoimmune collagen arthritis are of the IgG2a subclass. Activation of phagocytes occurs primarily through FcgRIII in both cases, but contribution of the classic or alternative pathway of complement activation differs (15, 16).

It is long recognized that expression of collagen type II specific immune complex arthritis can be enhanced by concomitant administration of recombinant proinflammatory cytokines or bacterial LPS. This LPS augmentation can be efficiently blocked with anti-TNF antibodies, highlighting that generation of TNF is crucial and elucidating yet another element of a TNF dependent co-stimulatory pathway in expression of arthritis. Moreover, it provides a potential link between environmental bacterial pressure and arthritis.

TNF and IL-1 in macrophage driven SCW arthritis

The above discussion was focused on arthritis driven by immunologic pathways. Other potential arthritogenic stimuli include the continued presence in the joint of bacterial cell wall fragments or bacterial DNA fragments bearing CpG motifs (17). These stimuli can directly activate synovial macrophages, with subsequent production of TNF and IL-1. As an example, streptococcal cell wall (SCW) fragments can induce arthritis upon direct injection into the murine knee joint. The acute joint swelling and early cell influx into the synovial membrane can be inhibited with anti-TNF treatment, using either neutralising antibodies or soluble receptors (TNF binding proteins). Intriguingly, anti-IL-1 treatment did not reduce these parameters. Just the opposite holds for another key parameter of the arthritic process, the inhibited chondrocyte synthetic activity in the articular cartilage, which together with enhanced proteolytic breakdown of cartilage matrix provokes pronounced cartilage des-
Chondrocyte-mediated cartilage repair function is fully normalized by anti-IL-1, whereas anti-TNF treatment was without effect (Fig. 2). These separate activities of TNF and IL-1 in this model argue for anti-TNF/IL-1 combination treatment, which was indeed shown to block both inflammation and cartilage damage and to provide the best overall protection (18). In line with the separate blocking effects of anti-TNF and anti-IL-1, IL-1 production appeared independent of TNF in this model (Fig. 3). IL-1 levels in synovial tissue were hardly reduced after anti-TNF treatment or in SCW arthritis in TNF deficient mice (19).

**IL-1 dependence of chronic, destructive SCW arthritis**

In an attempt to bring the model more close to the clinical situation, consecutive flares were induced by repeated injections of SCW fragments in the same knee joint, at weekly intervals. A model of chronic relapsing SCW arthritis ensued, which showed progressive cellular infiltration and joint damage. Remarkably, every flare still showed strong TNF dependence in terms of joint swelling. However, chronic cellular infiltration and cartilage damage were not inhibited by weekly dosing of anti-TNF antibodies or TNF soluble receptors. Moreover, induction of this model in TNF deficient mice even showed enhanced pannus formation and erosive changes.

In marked contrast, anti-IL-1 treatment had increasing effect on each consecutive flare (20), including significantly reduced swelling and full prevention of chronic synovial infiltrate and cartilage erosion. This protective effect was also seen in IL-1 deficient mice, herein identifying IL-1 as the dominant IL-1 subtype in this chronic process and demonstrating that repeated flare reactions render the cellular process in the synovial tissue more and more an IL-1 dependent phenomenon.

The most likely explanation for this shift is a growing contribution of anti-SCW directed T cell reactivity in the local infiltrate, as compared to the non-immune macrophage activation, in line with the recent findings of late IL-1 de-
dependent T cell reactivity in collagen arthritis as discussed above (12).

This is also compatible with the marked reduction of chronic SCW arthritis in lympho toxic deficient mice or RAG mice (unpublished observations), which lack proper lymphoid organization and immune responsiveness.

**Antigen induced arthritis and T cell flares**

This model is a severe, destructive joint inflammation elicited by the direct injection of an antigen into the knee joint of preimmunized animals and displays a mixture of immune complex and T cell driven arthritis. The acute, severe stage is hardly TNF and/or IL-1 dependent, suggesting substantial overkill by other mediators. Advanced arthritis and joint erosion is IL-1 dependent (21). Flares of the smouldering chronic arthritis which can be induced by rechallenge with small amounts of antigen are dominantly a local, T cell reactivation process and inflammation as well as joint destruction show strong IL-1 dependence (22).

**Combination therapy in rat adjuvant arthritis**

A final model that should be discussed is the classic adjuvant arthritis, which is induced in susceptible Lewis rats by immunization with complete Freund's adjuvant. It is considered as a pure T cell model, because the disease can be easily transferred with T cells and T cell immunomodulation greatly affects the course. The autoantigen which is recognized by the T cells has not been identified yet. Both TNF and IL-1 blocking were effective, compatible with a facilitating role of TNF in expression of threatening autoimmune reactivity and precipitation to the joint, together with a role of IL-1 in T cell reactivity and maturation.

Of great interest is the fact that marked synergy in arthritis suppression, including inflammation and bone erosion, was noted when inactive and moderately active doses of IL-1Ra and sTNFRI were applied together. Similar synergy was noted with such combined treatments in rat collagen arthritis (23, 24).

**General lessons from the animal model studies**

There is no doubt that anti-TNF treatment is efficacious in most RA patients. As such, of the arthritis model situations studied, the process of direct nonimmune macrophage activation with bacterial triggers seems to fit most with a dominant involvement of TNF. If immune elements come into play, IL-1 seems to become increasingly important. This holds both for immune complex arthritis and T cell driven arthritis (Fig. 4). When animals are immunized to induce autoimmune reactivity, resulting in circulating autoantibodies and autoreactive T cells, expression of arthritis depends on accumulation of these elements in the joint and this process is facilitated by TNF. Once the arthritic process is started and sustained by immune recognition of persistent (auto)-antigens, TNF dependency wanes, and IL-1 becomes dominant both in propagation of inflammation and induction of joint erosion. In all situations of macrophage activation with phlogistic stimuli, immune complexes or indirect triggering through activated T cells, TNF independent IL-1 production is evident.

**The clinical situation in RA patients**

It is a longstanding debate whether RA should be viewed primarily as a process of deranged synovial cell activation and/or an autoimmune process. If the only defect of RA is a deranged synovial cell, displaying too much TNF production, the therapeutic approach with TNF blockers is sufficient, since it will then automatically inhibit all TNF induced IL-1 production.

It is likely that the transition from a nonimmune process to an immune driven arthritis occurs rapidly in most animal models under heavy and continued antigenic pressure, whereas this process is more subtle in human RA and may take years to develop. Only upon loss of natural tolerance, immune elements come into play and potentially, the slow nature of such a process in RA may leave a window for early and effective anti-TNF treatment, together with methotrexate, to prevent such a transition.

If synovial biopsies of early RA patients are examined, considerable heterogeneity is a common finding. It is a personal experience that roughly 50% of the biopsies show significant TNF staining, whereas IL-1 is positive in all specimen. This dictates that at least in some patients IL-1 production is independent of TNF.

**Arguments for combination treatment with anti-TNF / IL-1**

Although treatment with anti-TNF antibodies or soluble TNFR is efficacious in the majority of RA patients, and also reduces joint erosions, it is too early to accept this as proof of a dominant TNF-IL-1 cascade in RA. Anti-TNF antibodies display cytotoxic effects, probably eliminating TNF-bearing cells and consequently also reducing IL-1 production in these or neighbouring cells. In addition, the soluble TNFR scavenges not only TNF but also lympho toxin, herein reducing T cell / IL-1 dependent pathways.

Recent clinical trials with anti-TNF identified that overall erosion was blocked, even in patients not showing an improvement of joint inflammation. More careful analysis revealed that healing of bone erosions occurred in some patients, implying progression in others.

The heterogeneity of the RA patient population, including variable degrees of immune involvement, and the lack of full insight in patients being TNF responders or not, argues for a combination treatment with TNF and IL-1 directed biologicals.

Anti IL-1 treatment in RA patients is not fully effective, using IL-1Ra as an approach, but it is hard to imagine sustained IL-1 receptor blockade under those conditions. In mice full protection is only achieved with continued dosing in Alzet minipumps or local gene therapy.

A further argument for combination treatment comes from the side effects recently noted with anti-TNF treatment in RA patients, ranging from recurrence of infections to expression of autoimmune phenomena such as encephalomyelitis. It was recently shown in
model studies that experimental encephalomyelitis was reduced in the beginning but exaggerated lateron in TNF deficient mice, with late development of marked anti-myelin T cell responses and identifying a homeostatic role of TNF in suppression (25). We made similar observations in the chronic relapsing SCW arthritis model, which appeared more severe in TNF deficient mice (unpubl observations). It suggests that complete TNF suppression should be avoided and combined treatment with intermediate doses of TNF and IL-1 blockers, reaching synergistic suppression of arthritis, seems warranted.

Final remarks

The recent observation of chronic, destructive arthritis in IL-1 receptor antagonist (IL-1Ra) deficient Balb/c mice provided a straightforward argument for the importance of IL-1 in arthritis (26). Similar to observations in TNF transgenic mice, IL-1 transgenic mice displayed overt arthritis (27). As discussed above, progressive erosive arthritis cannot be induced in IL-1 deficient mice, in contrast to its occurrence in TNF deficient mice. IL-1 deficient mice do not show enhanced infectious pressure. Apart from accepting IL-1 as a logic therapeutic target, further optimization of treatment can probably be achieved by targeting selective TNF receptor subclasses, involved in inflammation (25), leaving TNF immunosuppressive action intact.

References

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