

Interleukin-1 β and Interleukin-6 in arthritis animal models. Roles in the early phase of transition from the acute to chronic inflammation and relevance for human rheumatoid arthritis

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Abstract.

TNF α is the major target of the therapeutic approach in rheumatoid arthritis. Key issue in the approach to chronic arthritis is the understanding of the crucial molecules driving the transition from the acute phase to the chronic irreversible phase of the disease. In this review we analyzed five experimental arthritis animal models (AIA, AA, SCWA, CIA and SKG) considered as possible scenarios to interpret the biology of human rheumatoid arthritis. The SKG is strictly dependent on IL-6. In the others IL-1 β and IL-6, more than TNF α , appear to be relevant in driving the transition, suggesting that these should be the targets of an early intervention to stop the course towards the chronicity of the disease.

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Introduction.

The inflammatory cytokine tumor necrosis factor (TNF- α) is considered a pivotal mediator in chronic arthritis. Experiments performed 20 years ago provided the background for the development of anti-TNF strategies in human chronic arthritis: a) in *in vitro* culture of synovial fibroblasts from RA patients, Feldmann and Maini introduced the concept of cytokine hierarchy and showed that TNF, in this system, was “upstream” of other inflammatory cytokines, such as IL-1 β and IL-8 (1); b) consistently with this “up-stream” *in vitro* role of TNF, Kollias and co-workers showed that in vivo in mice the transgenic over-expression of TNF was sufficient to cause arthritis (2).

Other inflammatory cytokines may play a role in the induction and maintenance of chronic inflammation in synovial tissue. In this respect the most studied cytokines are IL-1 β and IL-6 that are both now targettable by specific inhibitors. In several experimental models TNF- α , IL-1 β and IL-6 are expressed very early on and play a key role in leading the inflammatory state.

A number of experimental arthritides are currently used. They are all characterized by synovial inflammation, cartilage damage and bone destruction. However, in all arthritides, there is a fundamental phase that needs to be critically examined: this is the early phase characterized by the transition from the acute to the chronic inflammatory phase. Animal models allow to evaluate the appearance and the changes in cytokine levels over time starting from the initiation of joint inflammation (i.e. experimental artificial induction). They may therefore provide data that help to unravel the role of each mediator in the different phases of the process leading to full-blown destructive joint inflammation. In this review we will discuss the available evidence in animal models on these 3 cytokines and their appearance during development and transition to a chronic phase in each model (see Figure 1 for a summary) and then relate these findings with what is known in early rheumatoid arthritis

Evidences from arthritis experimental models.

Antigen induced arthritis (AIA). AIA is an animal model for arthritis initially developed in rabbits and then applied also to mice and rats. The inducing antigen is methylated bovine serum albumin (mBSA) that, after systemic immunization, is then injected into the joint, and, therefore, AIA is a T cell-dependent immunological arthritis. In murine AIA it is possible to observe most histopathological characteristics of rheumatoid arthritis (RA), such as marked synovial lining hyperplasia, proliferation of sublining cells, infiltration of inflammatory cells, neovascularization, pannus formation, and articular cartilage destruction. In this model, one of the most evident sign is intense cartilage destruction together with prominent osteoclastogenesis. In IL-6 knock-out mice ,

arthritis is much milder and most importantly even though IL-1 β and TNF- α are expressed and are released during the inflammatory process, cartilage and bone destruction are markedly reduced (3). In rats with AIA synovial tissue mRNA levels of IL-1 β and IL-6 increase sharply by day 1, together with IL-2 and IFN- γ , whereas TNF- α increases later. A strong association can be found between joint swelling and expression of IL-1 β and IL-6 (4,5). Examination of draining lymphnodes revealed that the only cytokine increasing very significantly by 6 hours after induction of AIA was IL-6 (4). Also, in serum, IL-6 levels increase sharply by day 1, while IL-1 β and TNF peaks much later, with only a minor increase in TNF- α (5). These data suggest that IL-6 and IL-1 β are likely the driving cytokines in AIA. Data from knock-out (KO) mice support this hypothesis. IL-6KO mice treated with a standard protocol of immunization with mBSA did not develop joint swelling following intra-articular mBSA injection, nor revealed the characteristic joint lesions by histological examination (3,6). In addition, the lack of reactivity could not be compensated by the injection of IL-6 alone, but by the coinjection of IL-6 and IL-6R, and soluble gp130 could in fact decrease arthritis manifestations and the severity (7,8). A study comparing IL-6 with TNF- α KO mice showed that IL-6 deficiency conferred greater protection from AIA (9). To the best of our knowledge no studies are available for AIA in IL-1KO.

Adjuvant-induced arthritis (AA).

Adjuvant-induced arthritis is induced by *mycobacterium butyricum* suspended in mineral oil and injected subcutaneously in rats. A temporal analysis of the expression of cytokines showed that TNF- α was increased in the synovial lining starting from day 11, and in macrophages starting from day 18, while IL-6 appeared in the synovial lining, macrophages and endothelium from day 7. mRNA levels for the 3 cytokines are similarly upregulated at the time of appearance of joint swelling (day 10); however, TNF- α and IL-1 β levels peaked later (day 20 and day 16, respectively) than IL-6 (day 12-14). Synovial protein levels appeared from day 4 for IL-6, day 10 for TNF- α and day 18 for IL-1 β . When looking at serum levels, TNF- α and IL-1 β appeared from day 10, and IL-6 from day 25 (10). Therefore, in this model the early phases of the disease seems to be characterized by a systemic increase in IL-1 β and TNF- α , while the late phases by an increase in IL-6. However, locally in the inflamed joint increase in IL-6 occurs earlier than IL-1 β and TNF- α . A possible interpretation could be that IL-6 produced locally represents the mediator of the initial inflammation that is subsequently driven to a fully expressed systemic inflammation by IL-1 β and TNF- α . Ayer et al (11) examined the talar joint and the popliteal lymphnodes of rats with AA. In their study TNF- α and IFN- γ were significantly hyperexpressed at the mRNA level in popliteal lymphnode

before the onset of arthritis. At the synovial level the peak of IL-6 was at day 14, while the other cytokines peaked at later times (IL-1 β at day 16 TNF- α at day 20).

Therefore, in AA two studies have both shown that IL-6, together with IL-1, is the first molecule occurring at high levels in the synovial tissue in this T cell-dependent arthritis, suggesting that IL-1 β and IL-6 are involved in the early phase. However, their role in T cell recruitment and activation is not yet clarified in this model.

The streptococcal cell wall arthritis (SCWA).

In SCWA, synovitis is induced by local injection of SCW antigen directly into an ankle joint both in mice and rats, with maximal swelling at 24 hours. The initial response is reactivated by systemic (intravenous) challenge with SCW, which produces a more prolonged and severe inflammation confined to the joint previously injected with SCW. In this model also IL-1 α , TNF- α , and IL-6 are among the highest expressed cytokines a few hours after injection. However, when looking at the fold increase 4 hours and 3 days after the re-challenge, IL-1 β , TNF- α and IL-6 increased respectively by 22, 11.1 and 10 fold at 4 hours and by 5.7, 0 and 21.4 fold at day 3 (12). These data show that the three cytokines increase dramatically after the challenge, but the rechallenge (the one that induces the transition from acute to more persistent arthritis) is associated with a marked increase in IL-6 and IL-1 β . It comes of no surprise in this context that in this model the IL-17R signaling in radiation-resistant cells in the joint is required for turning an acute macrophage-mediated inflammation into a chronic destructive sinovitis (13).

Collagen induced-arthritis (CIA).

Collagen type II is one possible candidate autoantigen in human RA and it stimulates specific clonotypes that occur since the early phases of RA and during relapses of long standing RA (14). The data implicating collagen type II as an autoantigen came from the occurrence of arthritis in an animal model, the collagen induced arthritis (CIA) model. CIA is a polyarthritis induced by sensitization of susceptible strains of animals with type II collagen. Both humoral and cellular immune responses to collagen II are observed in sensitized animals and both components are involved in disease progression. In addition to joint inflammation and cartilage and bone damage, linkage of disease to genes residing in the histocompatibility locus and bone and autoreactive T and B cells, similar to the human disease, have led to consider CIA as one of the favourite models to study the inflammation occurring during arthritis development and to investigate drugs possibly active in human diseases.

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In this model on day 1, IL-6 shows a significant increase in the mRNA and protein level in the synovial tissue, IL-1 β and TNF- α show at day 1 an higher mRNA than protein expression. TNF- α shows a significant increase of the protein that occurs on day 4 and day 8. On day 11 which represents the peak of the arthritis, the protein levels are still significantly very high for IL-1 β , remain high though at lower levels than at day 1 for IL6, and reach the highest levels for TNF- α , though at significantly lower levels than for the other two cytokines (15). In this model therefore changes in IL-6 suggest that IL-6 plays a key role in the early phases of the disease and continues to be important during the transition to the more chronic phase. This conclusion is supported by data in the knock-out mice. Inactivation of the IL-6 gene in DBA/1J, mice led to complete protection from CIA, and this was associated with a reduced antibody response to type II collagen and the absence of inflammatory cells and tissue damage in knee joints (16). It is also interesting to note that the deletion of TNF- α gene does not confer complete protection from the occurrence of arthritis in CIA, while the knock-out of IL-1 fully protects against the onset of arthritis (17,18). Interestingly, early neutralization of IL-6 bioactivities, following immunization with Collagen type II leads to a significant reduction in Th17 cells and to a subsequent protection from CIA (19). Conversely, increased IL-1 signalling in conditional myeloid specific IL-1Ra deficient mice, results in increased IL-17 production by T cells associated with increased incidence and severity of the disease (20).

Spontaneous autoimmune arthritides: the SKG (Zap-70 mutation model) and K/BxN models.

These spontaneous models do not allow (with one partial exception, see below) to study the time course of cytokine appearance and changes overtime because of the absence of an artificially defined time of triggering (i.e. injection of eliciting antigen). The SKG strain of mice, a mutant on the BALB/c background, spontaneously develops T cell-mediated autoimmune arthritis, which clinically and immunologically resembles rheumatoid arthritis (RA) in humans. The strain harbours a recessive mutation of the gene encoding an SH2 domain of ζ -associated protein 70 (ZAP-70), a key signaling molecule in T cells. The arthritis is erosive, is RF (rheumatoid factor) and ACPA (anti-cyclic citrullinated peptide autoantibodies) positive and certainly is highly dependent on the development of CD4⁺ T cells secreting IL-17, a proinflammatory cytokine capable of recruiting and activating neutrophils and other inflammatory cells. The incidence and severity of SKG arthritis is significantly reduced when TNF- α , IL-1, or IL-6 genes are deleted, similar to the effects of anti-cytokine therapy in human RA.. However, the most clear-cut evidence is that the deletion of the IL-6 gene completely abolishes the occurrence of erosive arthritis, while IL-1 β gene deletion has an intermediate effect and TNF- α gene deletion abrogates only marginally the development of the

disease. Again IL-6 seems to be crucial for the initiation of the chronic phase and IL-1 β is the most compelling partner for sustaining the ongoing inflammation. Subsequently, IL-6 and TGF- β 1 locally produced seem to play a major role in the TH-17–IL-17 phase of this model of arthritis (21,22).

Another model of arthritis that presents similarities to human RA, is the K/BxN in mice. The K/BxN T cell receptor transgenic mice spontaneously develop an autoimmune disease with many of the clinical, histological, and immunological features of RA in humans (23, 24). The K/BxN arthritis is critically dependent on both T lymphocytes and B lymphocytes, which constitute an essential effector cell that produces arthritogenic antibodies. It might be viewed as a generic model of inflammatory arthritis mediated by antibodies and immune complex deposition in the joint. Indeed, transfer of antibodies from arthritic K/BxN mice to naïve animals (devoid of lymphocytes) causes arthritis. Using this transfer approach, sequential monitoring of mRNA cytokine levels in the joints in the early phases of the disease shows that TNF α , IL1 β and IL-6 appear in sequence. Development of arthritis requires IL-1, because mice deficient in the IL-1 receptor are refractory to disease (25). Moreover, TNF α is less strikingly important because a proportion of TNF- α deficient mice developed robust disease, and IL-6 deficiency does not modify the appearance and the course of the arthritis. These results imply that IL-6 does not appear to be involved in the effector phase of a purely antibody mediated joint inflammation, while IL-1 appears to be the pivotal driver.

Why is the transition phase so important ?

The transition from acute inflammation to chronic inflammation is the crucial phase in which a defensive innate response of the immune system turns into a potentially tissue damaging response (acquired or adaptive immune response). A fundamental step in this transition is the recruitment of the cells of the chronic phase. The recruitment of the immune cells of the adaptive phase of inflammation requires the synthesis and the release of chemochines attracting monocytes, T and B cells. In an acute inflammation, where the neutrophils (PMN) predominate, IL-6 favours PMN apoptosis. Apoptotic neutrophils have been shown to express new membrane antigens (phosphatidylserine, thrombospondin) that are recognized by various receptors on macrophages (scavenger receptors, phosphatidylserine receptor, α 5 β 3: vitronectin receptor), leading to further activation of their phagocytosis. Phagocytosis of apoptotic PMN by macrophages increases TGF β and MCP-1 secretion and decreases IL-8 production, leading to a chemokine shift favouring monocyte recruitment (26).

Before discussing the role of IL-6 in the transition phase it is necessary to discuss the peculiarities of the mode of functioning of the IL-6 receptor system, which is rather unique in cytokine biology.

IL-6 binds to a membrane IL-6 receptor (mIL-6R), that it not able to transduce signals. The IL-6/IL-6R complex binds to gp130, the signalling receptor subunit, and this triggers signal transduction by gp130. A soluble IL-6R (sIL-6R) is physiologically present at high concentrations in biological fluids and the IL-6/sIL-6R complex binds to gp130 and triggers signalling as efficiently as the full membrane IL-6R. Therefore, in contrast to the majority of the soluble cytokine receptors, sIL-6R behaves as an agonist (or as a co-cytokine). Moreover, since the expression of the membrane IL-6R is restricted to few cell types, while gp130 expression is almost ubiquitous, the presence of the sIL-6R widens the spectrum of target cells of IL-6. The signalling induced through sIL-6R is called trans-signalling (27). Interestingly also gp130 exists naturally in a soluble form, sgp130. A number of studies have shown that both natural sgp130 or genetically engineered Fc-sgp130 selectively block IL-6 trans-signalling mediated through the sIL-6R both in vivo and in vitro, pointing to the role of trans-signalling in several inflammatory conditions (28, 29), including AIA (7).

Both IL-1 β and IL-6, which are present since the early phases of several arthritis models, have in fact a rather unexpected role in leukocyte recruitment in vivo. Both IL-1 β and the IL-6/sIL-6R complex induce endothelial cells to secrete IL-8 and MCP-1, as well as to express adhesion molecules. Of interest IL-6 plus its soluble receptor (shed by apoptotic PMN) activate endothelial cells to produce more MCP-1 than IL-8 thus favouring monocyte recruitment (30,32). Indeed, Rabe et al have recently selectively blocked in vivo trans-signalling via the soluble IL-6 receptor through transgenic overexpression of soluble gp130 and have shown that in the air pouch model the production of MCP-1 and the subsequent recruitment of mononuclear cells is markedly impaired (33).

Another key aspect of this transition is represented by T and B cell recruitment and activation. In an elegant experimental model, the staphylococcus epidermidis peritoneal inflammation, a model that allows to study the transition from acute to chronic inflammation, it has been convincingly demonstrated that IL-6 and IL-6R selectively govern T cell infiltration by regulating chemokine secretion (CXCL10, CCL4, CCL5, CCL11, and CCL17) and chemokine receptor (CCR3, CCR4, CCR5, and CXCR3) expression on the CD3⁺ infiltrate (27). It has been clearly demonstrated that the essential role of IL-6 in T cell activation is to induce the cells to shift from G0 to G1, where they become more responsive to the small amounts of IL-2 induced by IL-1 (34, 35). This cooperation between IL-6 and IL-1 β may help to better understand the involvement of other molecules in the early phases, such as IL-17(36), which has been shown to be highly expressed in AA since day 5 (37). All together these data suggest that indeed the cooperation between IL-1 β and IL-6 represents a fundamental synergy to amplify the adaptive phase of the autoimmune inflammatory response.

Cytokines in early rheumatoid arthritis.

Intense efforts have been made over the last 20 years to unravel the cytokine features of RA. It has been reported that IL-1 β and IL-6, along with IL-2 and IFN- γ , but not TNF- α , is especially associated with the development of inflammation in plasma of pre-arthritis patients (38). Unfortunately only the already clinically manifested synovitides can be deeply studied, and in this respect very few studies are available. Starting from studies on cytokine levels in plasma, we have data suggesting that IL-1 β , IL-6 and IL-10 are the cytokines that are mostly increased in patients with disease duration shorter than 48 months and previously treated only with NSAIDs (39). This has been confirmed in a cohort of RA compared to undifferentiated arthritis (40). In general the synovial compartment from relatively early RA already show the same pattern of late RA (41). Canete et al examined the synovial tissue of patients with a disease duration shorter or longer than 12 months and were able to show that while TNF- α expression was comparable, the expression of IL-1 β and IL-6 as well as of TGF- β was more characteristic of the early phases of the disease (42). More recently the synovial fluid of patients with early synovitis was examined and the molecules that better distinguished the early synovitis (RA, crystal, etc) from controls with no inflammation (OA) were IL-1 β , IL-2 and IL-6; the cytokines distinguishing better the persistent synovitis of RA from the other synovitides were IL-2, IL-13 and IL-1 β (43). In summary the scanty available evidence suggest that indeed IL-1 β , IL-6 and possibly the Th2 related cytokines are those that better depicts the early phases of an ongoing arthritis such as RA.

Conclusions. The various experimental animal models suggest that the key driving molecules IL-1 β , TNF- α and IL-6 are certainly all involved in the early course of each arthritis model, yet the relative appearance is temporarily different for each cytokine in the very initial phases (Figure 1). In the majority of the models the key driver seems to be IL-1 β . Although few studies are available in the very early stages of a synovitis, the data in humans also suggest that two molecules, IL-1 β and IL-6 certainly play a major role. Indeed antagonizing IL-6 has been clinically very successful (44). Given the fundamental importance of the two cytokines in driving the early phases of inflammation and the transition from the acute to the chronic phase of the inflammatory process, it is conceivable to speculate that IL-1 β and IL-6, will become more and more key targets for an early intervention in human arthritis. Importantly, this will allow to assess whether antagonizing these targets in the very early phases of the disease could give us clearcut hints on whether there is a biological window of opportunity to reverse the cellular and molecular biology of the disease.

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FIGURE LEGEND

Synovial tissue expression of the mRNA for TNF- α , IL-1 β and IL-6 in arthritis animal models. We present the data of four models, except the SKG mode in which the IL-6 gene function is a required to have the disease: IL-6 gene knock-out fully abolishes the occurrence of arthritis, whereas the IL-1 β and TNF- α gene knock out mice only reduce the incidence of arthritis .

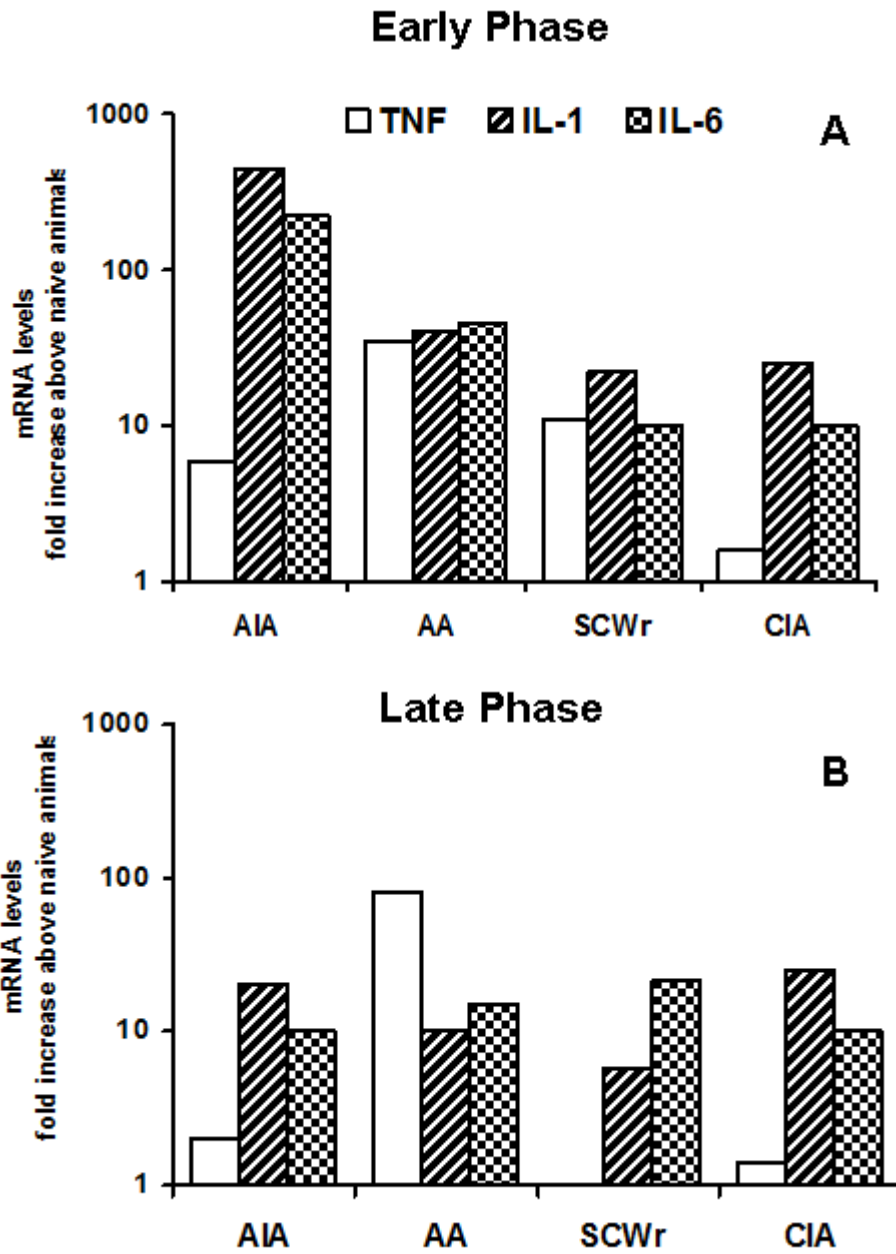
Panel A shows levels at the time of clinical appearance of arthritis (in the early phase) and panel B shows the levels at time full-blown arthritis (late phase). Data have been extrapolated from published papers (AIA Ref.4,5,6-AA Ref.8,9-SCWA Ref.10-CIA ref.13). Data are shown as fold increase compared to synovial tissue expression in naïve untreated control animals.

Early phase: day 1 after intra-articular injection for AIA; day 10-12 after induction (corresponding to first clinical appearance of joint swelling) for AA; day 1 after re-challenge for SCWA; day 12-15 after boosting (corresponding to the first clinical appearance of joint swelling) for CIA.

Late phase: day 6 after intra-articular injection for AIA; day 41-47 after induction (corresponding to clinical appearance of the maximum joint swelling) for AA; day 3 after re-challenge for SCWA; day 23-25 after boosting (corresponding to the maximum clinical appearance of joint swelling) for CIA

AIA, antigen-induced arthritis; AA, adjuvant arthritis; SCWr, streptococcal cell wall induced arthritis after rechallenge; CIA, collagen-induced arthritis.

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