

Interleukin-1 β and Interleukin-6 in Arthritis Animal Models: Roles in the Early Phase of Transition from Acute to Chronic Inflammation and Relevance for Human Rheumatoid Arthritis

Gianfranco Ferraccioli,¹ Luisa Bracci-Laudiero,^{2,3} Stefano Alivernini,¹ Elisa Gremese,¹ Barbara Tolusso,¹ and Fabrizio De Benedetti²

¹Division of Rheumatology, School of Medicine, Catholic University of the Sacred Heart, Rome, Italy; ²Ospedale Pediatrico Bambin Gesù, Rome, Italy; ³Institute of Neurobiology and Molecular Medicine, National Research Council, Rome, Italy

Tumor necrosis factor- α (TNF- α) is the major target of the therapeutic approach in rheumatoid arthritis. A 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 (antigen-induced arthritis, adjuvant-induced arthritis, streptococcal cell wall arthritis, collagen-induced arthritis and SKG) considered as possible scenarios to facilitate interpretation of the biology of human rheumatoid arthritis. The SKG model is strictly dependent on interleukin (IL)-6. In the other models, IL-1 β and IL-6, more than TNF- α , appear to be relevant in driving the transition, which suggests that these should be the targets of an early intervention to stop the course toward the chronic form of the disease.

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INTRODUCTION

The inflammatory cytokine tumor necrosis factor- α (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: in *in vitro* culture of synovial fibroblasts from rheumatoid arthritis (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 interleukin (IL)-1 β and IL-8 (1). Consistent with this upstream *in vitro* role of TNF, Kollias and colleagues showed that *in vivo* in mice the transgenic overexpression 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, which both can now be targeted by specific inhibitors. In several experimental models of the inflammatory state, TNF- α , IL-1 β and IL-6 are expressed very early on and play key roles.

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 must be critically examined. This is the early phase, which is characterized by the transition from the acute to the chronic inflammatory disease state. Animal models allow us to evaluate the appearance and the changes in cytokine levels over time, starting from the initiation of joint inflammation

(that is, experimental artificial induction). These models may therefore provide data that help us 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 discuss the available evidence in animal models regarding these three cytokines and their appearance during development and transition to a chronic phase in each model (summarized in Figure 1) and then relate these findings with what is known about early RA.

EVIDENCE FROM ARTHRITIS EXPERIMENTAL MODELS

Antigen-Induced Arthritis

Antigen-induced arthritis (AIA) is an animal model for arthritis that was initially developed in rabbits and then applied to mice and rats. The inducing antigen is methylated bovine serum albumin (mBSA), which, after systemic immunization, is injected into the joint. Therefore, AIA is a T-cell-dependent immunological arthritis. In murine AIA it is

Address correspondence and reprint requests to Gianfranco Ferraccioli, Director Division of Rheumatology, School of Medicine, Catholic University of the Sacred Heart, CIC, Via Moscatti 31, 00168, Rome, Italy. Phone: 39-06-3503654; Fax: 39-06-3503523; E-mail: gf.ferraccioli@rm.unicatt.it. Submitted May 31, 2010; Accepted for publication July 30, 2010; Epub (www.molmed.org) ahead of print August 2, 2010.

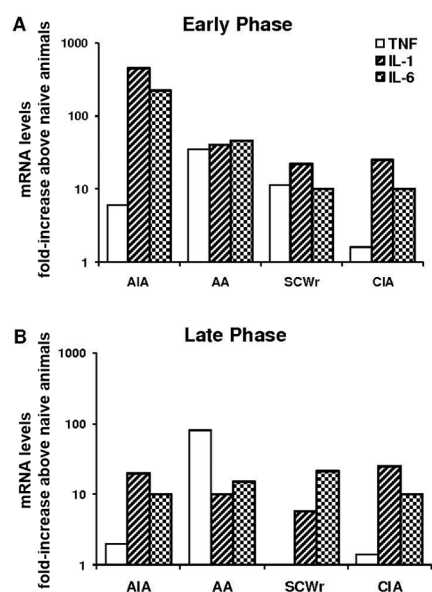


Figure 1. Synovial tissue expression of the mRNA for TNF- α , IL-1 β and IL-6 in arthritis animal models. We present the data from four models, except the SKG mode, in which the IL-6 gene function is required for the disease to occur, IL-6 gene knockout (KO) fully abolishes the occurrence of arthritis, whereas in IL-1 β and TNF- α gene KO mice the incidence of arthritis is only reduced. (A) shows levels at the time of the clinical appearance of arthritis (in the early phase), and (B) shows the levels at the time of full-blown arthritis (late phase). Data have been extrapolated from published articles: AIA (4,5,6), AA (8,9), SCWA (10) and CIA (13). Data are shown as fold-increase compared with synovial tissue expression in naïve untreated control animals. Early phase: day 1 after intraarticular injection for AIA; days 10–12 after induction (corresponding to first clinical appearance of joint swelling) for AA; day 1 after rechallenge for SCWA; days 12–15 after boosting (corresponding to the first clinical appearance of joint swelling) for CIA. Late phase: day 6 after intraarticular injection for AIA; days 41–47 after induction (corresponding to clinical appearance of maximum joint swelling) for AA; day 3 after rechallenge for SCWA; days 23–25 after boosting (corresponding to the maximum clinical appearance of joint swelling) for CIA.

possible to observe most histopathological characteristics of 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 signs is intense cartilage destruction together with prominent osteoclastogenesis. In IL-6 knockout (KO) mice, the arthritis is much milder. 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 increased sharply by day 1, together with IL-2 and interferon (IFN)- γ , whereas TNF- α increased later. A strong association has been found between joint swelling and expression of IL-1 β and IL-6 (4,5). Examination of draining lymph nodes revealed that the only cytokine that increased very significantly by 6 hours after induction of AIA was IL-6 (4). Also, IL-6 levels in serum increased sharply by day 1, whereas IL-1 β and TNF peaked 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 KO mice support this hypothesis. IL-6 KO mice treated with a standard protocol of immunization with mBSA did not develop joint swelling after intraarticular mBSA injection, nor did histological examination of these mice reveal the characteristic joint lesions (3,6). In addition, the lack of reactivity was not compensated for by the injection of IL-6 alone, but required the coinjection of IL-6 and IL-6R, and administration of soluble gp130 decreased the manifestation and the severity of the arthritis (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 have investigated for AIA in IL-1 KO mice.

Adjuvant-Induced Arthritis

Adjuvant-induced arthritis (AA) is induced by *Mycobacterium butyricum* suspended in mineral oil and injected subcutaneously into 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, whereas IL-6 appeared in the synovial lining, macrophages and endothelium from day 7. mRNA levels for the three cytokines were similarly upregulated at the time of the 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 . Investigation of serum levels revealed that 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 seemed to be characterized by a systemic increase in IL-1 β and TNF- α , and the late phases by an increase in IL-6. However, locally in the inflamed joint the increase in IL-6 occurred earlier than increases in IL-1 β and TNF- α . A possible interpretation of these results is that IL-6 produced locally acts as the mediator of the initial inflammation, which 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 lymph nodes of rats with AA. In their study, TNF- α and IFN- γ were significantly hyperexpressed at the mRNA level in popliteal lymph nodes before the onset of arthritis. At the synovial level the peak of IL-6 was at day 14, whereas the other cytokines peaked at later times (IL-1 β at day 16 TNF- α at day 20).

Therefore, for 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, which suggests that IL-1 β and IL-6 are involved in the early phase. However, the role of IL-1 β and IL-6 in T-cell recruitment and activation is not yet clarified in this model.

Streptococcal Cell Wall Arthritis

In mouse and rat models of streptococcal cell wall arthritis (SCWA), synovitis is induced by local injection of SCW antigen directly into an ankle joint, and max-

imal swelling occurs at 24 h after injection. 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 IL-1 α , TNF- α and IL-6 are also among the most highly expressed cytokines a few hours after injection. However, observations of the fold-increase 4 hours and 3 days after the rechallenge showed that IL-1 β , TNF- α and IL-6 increased respectively by 22-, 11.1- and 10-fold at 4 h and by 5.7-, 0- and 21.4-fold at day 3 (12). These data show that the three cytokines increased dramatically after the challenge, but the rechallenge (the one that induced the transition from acute to more persistent arthritis) was associated with a marked increase in IL-6 and IL-1 β . It comes as no surprise in this context that in this model the IL-17R signaling in radiation-resistant cells in the joint is required for the transition of an acute macrophage-mediated inflammation into a chronic destructive synovitis (13).

Collagen-Induced Arthritis

Collagen type II is one possible candidate autoantigen in human RA, and it stimulates specific clonotypes that occur in the beginning of the early phases of RA and during relapses of longstanding 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 have been 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, makes CIA one of the most useful models for study of the inflammation that occurs during arthritis

development and investigation of drugs that may be active in human diseases.

In this model, IL-6 on day 1 shows a significant increase in the mRNA and protein level in the synovial tissue, and IL-1 β and TNF- α at day 1 show 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 , and remain high, although at lower levels than at day 1, for IL-6, and reach the highest levels for TNF- α , although 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 from KO 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 the *TNF- α* gene does not confer complete protection from the occurrence of arthritis in CIA, whereas the KO of IL-1 fully protects against the onset of arthritis (17,18). Interestingly, early neutralization of IL-6 bioactivities, after immunization with collagen type II, leads to a significant reduction in Th17 cells and to subsequent protection from CIA (19). Conversely, increased IL-1 signaling 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

Spontaneous autoimmune arthritides models do not allow (with one partial exception, see below) study of the time course of cytokine appearance and changes over time because of the absence

of an artificially defined time of triggering (that is, injection of eliciting antigen). The SKG strain of mice, a mutant of the BALB/c background, spontaneously develops T-cell-mediated autoimmune arthritis, which clinically and immunologically resembles RA in humans. The strain harbors 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 positive for rheumatoid factor and ACPA (anticyclic citrullinated peptide autoantibodies) 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, whereas *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, locally produced IL-6 and transforming growth factor (TGF)- β 1 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. 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). K/BxN arthritis is critically dependent on both T lymphocytes and B lymphocytes, which constitute an essential effector cell that produces arthritogenic antibodies. K/BxN arthritis 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. With the use of this transfer approach, sequential monitoring of mRNA cytokine levels in the joints in the early phases of the disease showed that TNF- α , IL-1 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 did 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, whereas 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 chemokine-attracting monocytes, T and B cells. In an acute inflammation, in which the polymorphonuclear neutrophil (PMN) predominate, IL-6 favors 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, $\alpha 5\beta 3$: vitronectin receptor), leading to further activation of their phagocytosis. Phagocytosis of apoptotic PMN by macrophages increases TGF- β and monocyte chemoattractant protein 1 (MCP-1) secretion and decreases IL-8 production, leading to a chemokine shift that favors 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 is not able to transduce signals. The IL-6/IL-6R complex binds to gp130, the signaling 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 signaling 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 cocytokine). Moreover, because the expression of the membrane IL-6R is restricted to only a few cell types, whereas gp130 expression is almost ubiquitous, the presence of the sIL-6R widens the spectrum of target cells of IL-6. The signaling induced through sIL-6R is called trans-signaling (27). Interestingly, gp130 also exists naturally in a soluble form, sgp130. A number of studies have shown that both natural sgp130 and genetically engineered Fc-sgp130 selectively block IL-6 trans-signaling mediated through the sIL-6R both *in vivo* and *in vitro*, pointing to the role of trans-signaling in several inflammatory conditions (28,29), including AIA (7).

Both IL-1 β and IL-6, which are present from the early phases of several arthritis models, have rather unexpected roles 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 favoring monocyte recruitment (30–32). Indeed, Rabe *et al.* have recently selectively blocked *in vivo* trans-signaling 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, staphylococcus epidermidis peritoneal inflammation, a model that allows study of 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 elucidate the involvement of other molecules in the early phases, such as IL-17 (36), which has been shown to be highly expressed in AA beginning on day 5 (37). Taken together these data suggest that indeed the cooperation between IL-1 β and IL-6 represents a fundamental synergy that amplifies the adaptive phase of the autoimmune inflammatory response.

CYTOKINES IN EARLY RA

Intense efforts have been made during 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 , are especially associated with the development of inflammation in plasma of prearthritis patients (38). Unfortunately only the already clinically manifested synovitis 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 nonsteroidal anti-inflammatory drugs (39). This finding has been confirmed in a cohort of RA com-

pared with undifferentiated arthritis (40). In general the synovial compartment from relatively early RA already shows the same pattern as 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 although 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 (such as RA and crystal) from controls with no inflammation (OA) were IL-1 β , IL-2 and IL-6, and the cytokines that better distinguished the persistent synovitis of RA from the other synovitides were IL-2, IL-13 and IL-1 β (43).

In summary, the limited available evidence suggests that indeed IL-1 β , IL-6 and possibly the T-helper cell 2-related cytokines are those most likely to be important in the early phases of an ongoing arthritis such as RA.

CONCLUSION

Results of experiments in various animal models suggest that the key driving molecules IL-1 β , TNF- α and IL-6 are definitely all involved in the early course of each arthritis model, but the relative appearance is temporally different for each cytokine in the very initial phases (see Figure 1). In the majority of the models the key driver seems to be IL-1 β . Although few studies have investigated 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 that IL-1 β and IL-6 will increasingly become key targets for an early intervention in human arthritis. As important aspect of this process will be the assessment of

whether antagonizing these targets in the very early phases of the disease provides clear-cut hints on whether there is a biological window of opportunity to reverse the cellular and molecular biology of the disease.

DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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