Animal Models of Arthritis. What Have We Learned?

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ABSTRACT. Animal models of arthritis can be used to understand elements of the arthritic process in patients. Recent therapeutic approaches in patients with rheumatoid arthritis (RA) with biologics are based on initial findings in murine models of experimental arthritis, although final proof of concept must come from clinical studies. Animal models are powerful tools for studying pathologic changes in articular cartilage and bone in great detail, and can be used to evaluate mechanisms of erosive processes. Although in general more inflammation drives more destruction, the uncoupling of inflammation and erosion can be seen as well, and different mediators are involved in these processes. (J Rheumatol 2005;32 Suppl 72:7-9)

Key Indexing Terms:
ANIMAL DISEASE MODELS
RHEUMATOID ARTHRITIS
JOINT EROSION
PATHOLOGICAL PROCESSES
GENETICS

GENERAL REMARKS ON ANIMAL MODELS

No animal model fully resembles rheumatoid arthritis (RA). This is not surprising since models usually run for weeks to months, while the human disease takes years to develop and shows significant heterogeneity between patients. In general, RA is considered to be an autoimmune process, characterized by excessive autoantibody production, aggressive behavior of synovial cells, and abundant cytokine production. Animal models have shown that chronic, destructive arthritis, akin to that of joint structures, can be caused by multiple stimuli including persistent bacteria and viruses, as well as articular autoantigens. It is a matter of preference to consider common bacterial or viral elements, generally present in the joints, as exogenous or endogenous. Both common foreign proteins and classic autoantigens need tight regulation, and loss of tolerance against such stimuli is a crucial element in the arthritic process.

Given the level of complexity, the search for a dominant RA arthritogen is unrealistic. Animal models can at best be suggestive for putative arthritogens, but will not provide final answers. Instead, animal models can be used to identify common and selective pathways. They should be used to study aspects of human disease, and to understand common principles of chronicity of inflammatory processes, and destructive pathways involved in erosion of articular cartilage and bone. There is ample evidence that animal models have identified novel therapeutic targets. Moreover, animal models are useful in the first screening of promising novel treatments with biologicals. Subsequent trials in RA patients will provide ultimate proof of relevance in human disease, and distinct efficacy may indirectly prove the predictive value of animal models for particular outcomes.

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CYTOKINES AS NOVEL THERAPEUTIC TARGETS

The highly successful application of anti-tumor necrosis factor (TNF) therapy in patients with RA has been based on thorough studies in a range of animal models. Elegant studies in the early 1990s were done by George Kollias and colleagues showing that transgenic or dysregulated overexpression of TNF caused polyarthritis in mice[1]. At the same time, several groups showed in various arthritis models that neutralization of TNF, using antibodies or scavenging receptors, ameliorated arthritis and concomitant joint destruction[2]. This provided the basis for preparation of similar blocking agents to treat RA patients. Nowadays the success of anti-TNF therapy is seen as a major breakthrough in the understanding of master cytokines in arthritis and the crucial relevance of TNF in this disease. It also paved the way for similar approaches with optimized biological response modifiers, including engineered antibodies and soluble receptors.

Intriguingly, at the same time, interleukin 1 (IL-1) was identified as a crucial mediator in experimental arthritis models, showing higher potency compared to TNF, in particular as a driving force of cartilage erosion. Blocking of IL-1 was more effective than TNF neutralization in murine collagen arthritis[2-4]. In addition, IL-1 was identified as the secondary mediator of TNF in TNF transgenic mice, since this form of arthritis could be blocked with antibodies against the IL-1 receptor[5]. Thus animal model studies provided compelling evidence for IL-1 as a promising therapeutic target. Unfortunately, subsequent studies in clinical trials were done with soluble IL-1R type II, which showed poor efficacy. Later, it was found to show high affinity to bind to IL-1Ra, the natural receptor antagonist of IL-1, thereby scavenging this endogenous inhibitor and providing some explanation for the poor efficacy. Next, trials were done with IL-1Ra. This molecule showed better efficacy, but the effect was still less impressive compared with anti-TNF therapy. One conclusion might be that IL-1 is less important in humans compared to mice. However, IL-1Ra has poor pharmacokinetics, and a scavenging receptor antagonist has to be present in sufficient quantities all the time to prevent IL-1 receptor interaction. This puts a high demand on such

an inhibitor, and indeed animal models showed poor efficacy of IL-1Ra even after multiple dosing per day. Indeed, IL-1Ra treatment only achieved consistent suppression of collagen arthritis when applied in osmotic minipumps, which created sustained high levels of the inhibitor. In addition, great efficacy was shown when IL-1Ra was overexpressed in murine arthritic joints with local gene therapy[6,7]. Before accepting that animal models have given conflicting information and IL-1 is not a good target in human RA, proper blocking studies have to be done in RA patients with high quality anti-IL-1 molecules. Several trials with engineered IL-1 traps and IL-1 neutralizing antibodies have just started and will provide the final answer.

CYTOKINE DOMINANCE IN VARIOUS FORMS AND STAGES OF ARTHRITIS

TNF appears to be a driving cytokine in the early swelling and synovial cell influx observed in acute stages of all forms of experimental arthritis, including macrophage-driven inflammation and immune-dependent processes. However, IL-1 seems equally crucial in immune-mediated arthritis, such as immune complex and T cell-driven arthritis. As an example, repeated challenge with bacterial fragments induces a chronic relapsing arthritis in mice, which starts as a TNF-dependent macrophage-driven arthritis, but becomes more T cell-dependent with every rechallenge, and IL-1 drives late-cell infiltration and erosion[3]. In addition, IL-17, which is a T cell-derived cytokine, becomes a significant player as well. Of interest, this chronic, erosive model can be induced in TNF-deficient mice, in the full absence of TNF[3,8]. In accord with these observations, arthritis induced by TNF overexpression in TNF-transgenic mice is immune-independent, and is still seen in the background of a RAG-1-deficient mouse, lacking functional T and B cells. In marked contrast, Horai and Iwakura and colleagues recently developed a novel spontaneous arthritis model by deleting IL-1Ra. Such mice have uncontrolled IL-1 activity and appear to develop a T cell-dependent erosive arthritis that is also IL-17-dependent[9,10]. These observations illustrate that different cytokines can be more or less important under defined experimental conditions, and this may provide insight into heterogeneity in responsiveness of various RA patients to anticytokine treatment.

In addition, we have recently shown that cytokine dependency in a particular arthritic condition can be shifted by the presence of co-stimuli. As an example, murine immune complex arthritis is strongly IL-1-dependent[11,12], but loses this dependency when co-stimuli such as lipopolysaccharide or bacterial cell wall fragments are added, which activate Toll-like receptors (TLR). It was found that the cellular signaling pathways of TLR and the IL-1 receptor, which drive the production of inflammatory mediators, show considerable overlap, thus creating redundancy of the IL-1R pathway and making the arthritis IL-1-independent. As another example, murine collagen-induced arthritis, which is a mixture of immune complex and T cell-driven arthritis, is strongly IL-1-dependent, but this IL-1 dependency can be overruled when the T cell cytokine IL-17 is abundantly overexpressed in such arthritic joints. These examples reflect extremes in defined animal model settings, but are indicative of the relative dominance of one or the other cytokine in a particular arthritic process, which can be shifted by the presence of additional arthritogenic triggers. Whether similar conditions apply to human RA remains to be identified. If so, it may offer an additional explanation for variation in patient responsiveness to therapy.

UNCOUPLING OF INFLAMMATION AND JOINT EROSION

In general, it is believed that the degree of joint inflammation determines the degree of erosion of cartilage and bone. Cytokines like TNF, IL-1, and IL-17 drive joint inflammation, but also trigger osteoclast activation with subsequent erosion of bone, as well as activation of catabolic pathways in articular chondrocytes. It is now established that the recently discovered molecule RANKL is upregulated by TNF, IL-1, and IL-17 in osteoblasts and activated synovial cells. Through interaction with its receptor RANK, expressed on osteoclasts, it drives bone erosion. Elegant studies in RANKL and RANK knockout mice proved the crucial role of this pathway, since bone loss was completely blocked in arthritis in these mice, despite continuing inflammation[13-15]. In addition, a natural inhibitor of the RANKL-RANK interaction was identified, which was called osteoprotegerin (OPG). Recent studies in mouse arthritis models with OPG revealed the impressive potential of OPG to selectively block bone erosion, leaving the inflammatory process undisturbed[16-18]. OPG may provide an alternative therapeutic approach to control bone erosion, apart from cytokine blocking. A positive feature of downstream blocking of bone erosion with OPG might be that it leaves cytokines, and therefore our resistance to infections, intact.

Similar efficacy to selectively control bone and cartilage erosion was shown in animal models after local treatment with the modulatory cytokine IL-4. This Th2-derived cytokine reduced RANKL and upregulated OPG, which may explain its bone-protective effect[19]. In addition it upregulated defense mechanisms in chondrocytes and reduced the catabolic impact of IL-1 and IL-17, thereby providing protection against cartilage erosion. Apart from identification of novel therapeutic modalities in these animal model studies, these experiments also provided insight into the critical balance of destructive and controlling mediators. The erosive nature of an arthritic process in an inflamed joint is determined not only by the level of destructive cytokines, but also by the presence of counteracting mediators.

FUTURE DIRECTIONS

Genomics technology with gene chips provides the opportunity to do gene expression analysis in inflamed synovial tissue of RA patients as well as a range of animal models. Careful comparison of expression patterns before and after therapy may help to identify the nature of processes in RA patients and may also improve the predictive value of certain animal models. Gene chip technology will certainly identify novel genes, as well as potential novel targets. Animal models will be pivotal and powerful tools for target validation, using viral small interfering RNA technology *in vivo* to silence novel genes, whose functions remain to be understood.

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