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In Vivo Models of Inflammation

2nd Edition, Volume I

Christopher S. Stevenson
Lisa A. Marshall
Douglas W. Morgan

Editors

Birkhäuser Verlag
Basel · Boston · Berlin

Editors

Christopher S. Stevenson
Novartis Institutes for Biomedical Research
Respiratory Disease Area
Novartis Horsham Research Centre
Wimblehurst Road
Horsham, West Sussex
United Kingdom

Lisa A. Marshall
Johnson and Johnson PRD
Welsh & McKean Rd
Spring House, PA 19477
USA

Douglas W. Morgan
Portfolio, Program and Alliance
Management
BiogenIdec
14 Cambridge Center
Cambridge, MA 02142
USA

A CIP catalogue record for this book is available from the Library of Congress, Washington D.C., USA

Bibliographic information published by Die Deutsche Bibliothek
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie;
detailed bibliographic data is available in the internet at http://dnb.ddb.de

ISBN-10: 3-7643-7519-1 Birkhäuser Verlag, Basel – Boston – Berlin
ISBN-13: 978-3-7643-7519-5 Birkhäuser Verlag, Basel – Boston – Berlin

Part of Springer Science+Business Media
Printed on acid-free paper produced from chlorine-free pulp. TCF ∞
Cover design: Markus Etterich, Basel
Cover illustration: see p. 44; with friendly permission of Leo Joosten
Printed in Germany
ISBN-10: 3-7643-7519-1
ISBN-13: 978-3-7643-7519-5

e-ISBN-10: 3-7643-7520-5
e-ISBN-13: 978-3-7643-7520-1

9 8 7 6 5 4 3 2 1

www.birkhauser.ch

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List of contributors

Adriana Albini, Dept of Translational Oncology, Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy; e-mail: adriana.albini@istge.it

Karen Anderson, Novartis Institutes for Biomedical Research Inc, 250 Massachusetts Ave, Cambridge, MA 02139, USA; e-mail: karen.anderson@novartis.com

Nicole Avitahl-Curtis, Novartis Institute for Biomedical Research, 100 Technology Square, Cambridge, MA, USA; e-mail: nicole.avitahl@novartis.com

Roberto Benelli, Dept of Translational Oncology, Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy; e-mail: roberto.benelli@istge.it

Susan Brain, King's College London, Cardiovascular Division, Guy's Campus, London SE1 1UL, UK; e-mail: sue.brain@kcl.ac.uk

Jeffrey Everitt, GlaxoSmithKline Pharmaceuticals, LAS, 709 Swedeland Rd, King of Prussia, PA 19406, USA; e-mail: jeffrey.x.everitt@gsk.com

Guido Frumento, Dept of Translational Oncology, Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy; e-mail: guido.frumento@istge.it

Bajin Han, GlaxoSmithKline, Research Triangle Park, NC 27709, USA; e-mail: bajin.8.han@gsk.com

Takuya Ikeda, GlaxoSmithKline, Tsukuba Research Laboratories, 43 Wadai, Tsukuba 300-4243, Japan; e-mail: takuya.ikeda@gsk.com

Bruce D. Jaffee, Novartis Institutes for Biomedical Research Inc, 250 Massachusetts Ave, Cambridge, MA 02139, USA; e-mail: bruce.jaffee@novartis.com

Leo A.B. Joosten, Rheumatology Research and Advanced Therapeutics, Department of Rheumatology, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands; e-mail: l.joosten@reuma.umcn.nl

Naoko Kagiyama, Central Institute for Experimental Animals, 1430 Nogawa, Miyamae, Kawasaki 216-0001, Japan; e-mail: naoko-kagiyama@msh.biglope.ne.jp

Nandhakumar Kanagarajan, Division of Gastroenterology and Hepatology, Drexel University College of Medicine, Philadelphia, USA;
e-mail: nandhak77@yahoo.com

Karen F. Kozarsky, Biopharmaceuticals, Center of Excellence for Drug Discovery, GlaxoSmithKline, 709 Swedeland Road, King of Prussia, PA 19406, USA; e-mail: karen.f.kozarsky@gsk.com

Virginia B. Kraus, Duke University Medical Center, Durham, NC 27710, USA; e-mail: vbk@duke.edu

Margaret S. Landi, GlaxoSmithKline Pharmaceuticals, LAS, 709 Swedeland Rd, King of Prussia, PA 19406, USA; e-mail: margaret.s.landi@gsk.com

Joanne B. Morris, GlaxoSmithKline Pharmaceuticals, LAS, 709 Swedeland Rd, King of Prussia, PA 19406, USA; e-mail: joanne.b.morris@gsk.com

Matthias Müller, Novartis Pharma AG, WSJ-386.409, 4002 Basel, Switzerland; e-mail: matthias.mueller@novartis.com

Narasim S. Murthy, Associated Radiologists, PA, 322 E. Antietam Street, Suite 106, Hagerstown, MD 21740, USA; e-mail: duhidunno1@yahoo.com

Sreekant Murthy, Division of Gastroenterology and Hepatology and Office of Research, Drexel University College of Medicine, Philadelphia, USA;
e-mail: sm53@drexel.edu

Antonio Musarò, Department of Histology and Medical Embryology, CE-BEMM and Interuniversity Institute of Myology, University of Rome "La Sapienza", Via A. Scarpa 14, 00161 Rome, Italy; e-mail: antonio.musaro@uniroma1.it

Tatsuji Nomura, Central Institute for Experimental Animals, 1430 Nogawa, Miyamae, Kawasaki 216-0001, Japan; e-mail: tnomura@ciea.or.jp

Douglas M. Noonan, Dept of Clinical and Biological Sciences, University of Insubria, Varese, Italy; e-mail: douglas.noonan@uninsubria.it

Elisabeth Papazoglou, School of Biomedical Engineering, Drexel University, Philadelphia, USA

Nadia Rosenthal, European Molecular Biology Laboratory, Mouse Biology Unit, Monterotondo, 00016 Rome, Italy; e-mail: rosenthal@embl-monterotondo.it

Lisa R. Schopf, Abbott Bioresearch Center, 100 Research Drive, Worcester, MA 01605, USA; e-mail: lisa.schopf@abbott.com

Stephen A. Stimpson, GlaxoSmithKline, Research Triangle Park, NC 27709, USA; e-mail: stephen.a.stimpson@gsk.com

Wim B. van den Berg, Rheumatology Research and Advanced Therapeutics, Department of Rheumatology, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands; e-mail: w.vandenberg@reuma.umcn.nl

Preface to the first edition

The purpose of this volume in the series *Progress in Inflammation Research* is to provide the biomedical researcher with a description of the state of the art of the development and use of animal models of diseases with components of inflammation. Particularly highlighted are those models which can serve as *in vivo* correlates of diseases most commonly targeted for therapeutic intervention. The format is designed with the laboratory in mind; thus it provides detailed descriptions of the methodologies and uses of the most significant models. Also, new approaches to the development of future models in selected therapeutic areas have been highlighted. While emphasis is on the newest models, new information broadening our understanding of several well-known models of proven clinical utility is included. In addition, we have provided coverage of transgenic and gene transfer technologies which will undoubtedly serve as tools for many future approaches. Provocative comments on the cutting edge and future directions are meant to stimulate new thinking. Of course, it is important to recognize that the experimental use of animals for human benefit carries with it a solemn responsibility for the welfare of these animals. The reader is referred to the section on current regulations governing animal use which addresses this concern.

To fulfill our purpose, the content is organized according to therapeutic areas with the associated models arranged in subcategories of each therapeutic area. Concepts presented are discussed in the context of their current practice, including intended purpose, methodology, data and limitations. In this way, emphasis is placed on the usefulness of the models and how they work. Data on activities of key reference compounds and/or standards using graphs, tables and figures to illustrate the function of the model are included. The discussions include ideas on a given model's clinical correlate. For example, we asked our contributors to answer this question: How does the model mimic what is found in human clinical practice? They have answered this question in many interesting ways.

We hope the reader will find the information presented here useful for his or her own endeavours investigating processes of inflammation and developing therapeutics to treat inflammatory diseases.

October, 1998

Douglas W. Morgan
Lisa A. Marshall

Preface to the second edition

Since our first edition of "*In Vivo* Models of Inflammation" published in 1999, there has been amazing progress, and an abundance of exciting new information in inflammation research: new technologies, new therapeutics, new understanding of inflammatory processes, … and on and on, have emerged in the past 6 years. Supporting all of this are the fundamentals of inflammation research, i.e., the animal models, known mechanisms, and therapeutic standards, that have continued to provide the basis for generating these advances. Given the great progress, we have chosen to provide a second edition to our original text.

The second edition of "*In Vivo* Models of Inflammation" comes to you in two volumes and provides an update of the models included in first edition with expanded coverage and more models. Again, these volumes emphasize the standard models regarded as the most relevant for their disease area. The intent is to provide the scientist with an up-to-date reference manual for selecting the best animal model for their specific question. Updates on previously described models are specifically focused on references to any additional pharmacology that has been conducted using these systems. The sections on arthritis models have been expanded and now include models relating to osteoarthritis. New areas described herein include models of neurogenic, cancer, and vascular inflammation. Additionally, coverage of *in vivo* technologies includes updates on transgenic and gene transfer technologies, and has also been expanded to include chapters on stem cells and nanotechnologies.

The second edition continues to emphasize that conducting *in vivo* research carries with it a great responsibility for animal respect and welfare. The coverage of this concern has been extended to include chapters describing current regulations in the United States, the United Kingdom, and Japan.

The ultimate aim of the second edition is to provide current best practices for obtaining the maximum information from *in vivo* experimentation, while preserving the dignity and comfort of the animal.

We hope the information provided here helps in advancing the reader's endeavors in investigating processes of inflammation and in developing therapeutics to treat inflammatory diseases.

May, 2006

Christopher S. Stevenson
Lisa A. Marshall
Douglas W. Morgan

Rat models of arthritis: Similarities, differences, advantages, and disadvantages in the identification of novel therapeutics

Lisa R. Schopf, Karen Anderson and Bruce D. Jaffee

Millennium Pharmaceuticals, Cambridge, MA, USA

Introduction

The aim of this chapter is to update and expand the information reviewed by Carlson and Jacobson on the topic of rat models of arthritis [1]. Animal models of rheumatoid arthritis (RA) have been extensively used for many years in the evaluation of anti-arthritic agents [2–4]. The most widely used model, adjuvant-induced arthritis (AA) in rats, is discussed in detail here [4–6]. Another common model, which was not included in the first edition and is perhaps more relevant to human RA in terms of cartilage damage, is collagen-induced arthritis (CIA) in the rat [3, 7, 8], and is also outlined here. These two models are compared with a relatively new model, monoarticular streptococcal cell wall–induced arthritis (SCW) in the rat [1, 3, 9, 10]. All of these models share key features related to human RA that make them critical tools in drug development. They have provided information regarding genetic predisposition, prominent cell types, protein and molecular mediators involved in the immunological and inflammatory processes that leads to arthritic pathology.

Historical background

The first reported observation that complete Freund's adjuvant (CFA) could induce polyarthritis in rats was demonstrated by Stoerk and colleagues in 1954 [11] using spleen extracts emulsified in CFA. Shortly thereafter, Pearson showed that CFA alone could induce arthritis in rats [12]. Over the next decade, the AA rat model was used to test a variety of anti-arthritic therapies such as steroids and nonsteroidal anti-inflammatory drugs (NSAIDs) [1, 4]. More recently, this model has been used to assess immunomodulatory drugs such as methotrexate and cyclosporine A as well as therapies designed to block COX-2, TNF-α or IL-1 [13–21]. Overall, the AA model has been the most extensively used arthritic rat model by the pharmaceutical industry, and has an excellent track record for predicting both activity and toxicity [19].

In Vivo Models of Inflammation, Vol. I, edited by Christopher S. Stevenson, Lisa A. Marshall and Douglas W. Morgan

The CIA model was first described in 1977 by Trentham and colleagues, and has since gained favor by providing clues into the pathogenesis of arthritis and related disorders as well as its predictive value for testing anti-rheumatic therapies [7, 8]. This model has been used to evaluate NSAIDs, methotrexate and cyclosporine A as well as newer therapies, which block TNF-α and/or IL-1 [19, 22–27]. Although there are more data using the AA model, the rat CIA model has also proven to have predictive value for many current therapies and tends to be favored when examining protection against cartilage destruction because the lesion is more comparable to human RA than in the AA model [19].

Although we have known since the 1950s that injections of streptococcal cell wall components or more specifically covalent complexes of peptidoglycan and polysaccharide (PG-PS) from group A streptococci can induce rheumatic-like lesions, the monoarticular SCW model which is described in this chapter, was not developed until the mid-1980s, and has not been routinely used for pharmacological screening [1, 28–31]. However, in this review we provide details on methods and disease parameters such as joint swelling, histopathology, gene expression, serum acute-phase proteins and cartilage and bone markers. We also report efficacy determinations using this model to evaluate some of today's current therapeutics.

Drug therapies

There has been and continues to be extensive research in the area of drug development to treat human RA, but it is not the intention of this review to fully recount or explore all of these efforts [1–3, 32–36]. However, we have provided information on some of the more commonly used RA therapies and their efficacy in the three rat arthritic models (Tab. 1). This table focuses solely on therapeutic dosing regimes, although there is a vast amount of literature that explores prophylactic treatments as well [1, 2, 13, 14, 22, 23, 30, 37–44].

When patients first present with symptoms, the primary care physician will typically suggest the use of NSAIDs to provide some relief from pain and stiffness. Two examples of commonly used NSAIDs are ibuprofen and naproxen in Table 1, where we provide efficacy data in the rat models of arthritis. The main disadvantage to these treatments is that they only provide partial relief from pain and stiffness, but do not radically change the course of disease progression, as also predicted using the animal models (AA and CIA) [40]. They are typically only tolerated for short periods of time, after which patients can experience any number of gastrointestinal toxicity problems. Sometimes, corticosteroids are prescribed at this early stage, but, despite their potent anti-inflammatory action, they too have many dose-dependent side effects. Again, both the AA and CIA rat models predicted this outcome observed in patients [2]. Alternatively, corticosteroids such as prednisone are used

Table 1 - Efficacy of standard RA drugs using therapeutic dosing regimes in rat models of arthritis: AA, CIA and monoarticular SCW

Drug class	AA ED_{50} (mg/kg)	Ref.	CIA ED_{50} (mg/kg)	Ref.	SCW ED_{50} (mg/kg)	Ref.
NSAIDs						
Ibuprofen	75[a]; 65[a]	[123, 124]	49% @ 25 mg/kg[e]	[42]	ND	
Naproxen	7[a]	[125, 126]	ND		ND	
Corticosteroids						
Prednisolone	0.3[a]	[124]	ND		ND	
Dexamethasone	0.005[a]; 0.01[b]	[1]; MPI	0.01[f]	MPI	0.01[i]	MPI
DMARDS						
Methotrexate	Inactive[a,b]	[1]; MPI	0.1[g]		56% @ 0.1–0.5[i]	MPI
Cyclosporine A	2.4[c]	[127]	Enhanced[e,f]	MPI; [42, 43]	18[i]	MPI
Leflunomide	53% @ 32 mg/kg[d]	[128]	ND		ND	
Biologics						
Etanercept	ND		11[h]	MPI	<5[j]	MPI

ND, limited or no data available
MPI, unpublished data from Millennium Pharmaceuticals, Inc.
[a] *Once a day oral dosing from days 12 to 29*
[b] *Once a day oral dosing from days 10 to 19*
[c] *MWF oral dosing (6 doses)*
[d] *Once a day oral dosing from days 15 to 24*
[e] *Once a day oral dosing from days 14 to 27*
[f] *Once a day oral dosing from days 12 to 20*
[g] *Once a day oral dosing from days 6 to 21*
[h] *s.c. dosing days 12, 15, 18*
[i] *Once a day oral dosing from days 21 to 24*
[j] *s.c. dosing day 21*

at low doses (< 10 mg/day) during acute flares [34]. Therefore, the primary care physician, or at this point a rheumatologist, will plan to prescribe a disease-modifying antirheumatic drug (DMARD), such as the examples given in Table 1 of methotrexate, cyclosporine A or the newer drug leflunomide. One major drawback of DMARDs is that they only begin to demonstrate efficacy after several weeks of

therapy, and remission is rare, only 20–25% of patients [33]. Out of these examples, methotrexate is clearly the gold standard of care, and has been used since the 1950s. Patients on methotrexate often take a folic acid supplement to decrease toxic side effects. The most significant disadvantage to methotrexate therapy is that up to two thirds of RA patients on methotrexate will have an inadequate response to monotherapy. In the animal models, methotrexate is most efficacious when administered prophylactically and during the developing disease. This may be due to the need for prolonged dosing *in vivo*, analogous to what is observed clinically [2]. Methotrexate is given weekly to patients, which is difficult to mimic in the rat models due to the accelerated nature of disease progression in animals. Cyclosporine A is used as a common comparator drug in pharmaceutical drug evaluation because of its directed effects on T cells, but its relative toxicity has precluded its widespread use in RA patients. In fact, it has been demonstrated that in the rat CIA model, when given therapeutically, cyclosporine A can actually enhance disease, perhaps due to an alteration in the sensitive balance of helper *versus* suppressor T cells [43]. Leflunomide has only shown relatively modest efficacy in patients when used alone, and it also suffers from safety and tolerability issues, mostly related to elevations in liver enzymes and gastrointestinal issues. In the animal models, leflunomide administration has been most effective when given prophylactically [38, 45].

The most widely used biologics on the market inhibit the action of TNF-α; there are three products available: infliximab, adalimumab, and etanercept. We have provided animal data using etanercept in Table 1, and it is important to keep in mind that this product has been specifically designed to inhibit the action of human TNF-α and its differential potency to rat TNF-α is not known. The major disadvantages of this therapy are the high cost (a third of patients will not respond), and the risk of opportunistic infection.

Although we have been quick to point out the disadvantages of these selected therapies, they are currently the best the field has to offer, and they do provide relief and in some cases disease modifying activity to many patients. More recently, a combination therapy approach has provided added benefit to patients [14, 16, 19, 24, 25, 32, 36, 45–47]. Overall, the treatment of RA has dramatically improved over the last decade, with early diagnosis, and with new therapies on the horizon that will offer continued progress in caring for these patients.

Disease initiation and pathogenesis

Most of the RA therapeutics currently in use have been evaluated using rat arthritis models, which is a testament to the similarity in mechanisms that drive disease development. Although many pathological features are the same, the rat models progress much more rapidly, with acute, severe inflammation and dramatic changes in bone that include both formation and resorption [2, 48, 49].

Susceptibility of rats to the development of arthritis is dependent on a variety of factors including strain, environment, age, and gender. Also, as with many disease states, susceptibility may not be attributed to any one factor alone. For an excellent review of individual strain susceptibility, refer to the chapter by Carlson and Jacobson in the first edition of this book [1]. Simply stated the specific genetic basis of differing susceptibility is not known, but apparently it is not just a matter of MHC expression. For example two different strains, which share the same MHC, can exhibit different susceptibilities. As related to environmental influence, the role of endogenous flora is illustrated by the differing susceptibility of F344 rats to AA and SCW. More specifically, animals maintained in a conventional facility are resistant to the models, whereas animals in germ-free housing are susceptible [50, 51]. We and others have observed that very young and much older animals are relatively resistant to arthritis development [4, 52]. The short life span of rodents compared to humans thereby makes age dependency a difficult parameter to correlate. However, as in human RA, females are more prone to arthritis development in the rat models of CIA and SCW, although there is equal gender susceptibility to AA. Lewis rats are an example of a strain that is susceptible to all the models discussed here. Susceptibility in this strain is perhaps related, at least in part, to a defect in their hypothalamo-pituitary-adrenal axis and resultant reduced ability to suppress inflammation [53]. We routinely use female Lewis rats approximately 2 months of age (or 150–170 g) in all of our studies, as this allows for comparison of the disease process between models, and for better assessment of efficacy of known and novel therapeutics without additional variables associated with gender and strain differences [54].

All three models discussed here are initiated by the introduction of foreign antigen, either of bacterial origin as in AA (mycobacterial) and SCW (PG-PS 100P from *Streptococcus*) or using xenogeneic type II collagen (in this case bovine) in CIA. In general the antigens are rapidly redistributed from the site of introduction, and there is subsequently a T cell response. T cells have been shown to play an essential role in the development of all three models [55]. The supporting evidence includes lack of disease development in athymic rats, transfer of disease to naïve animals using lymph node cells or thoracic duct cells from arthritic rats, and efficacy of anti-T cell therapeutics including antibodies or cyclosporine A [56–61]. In human RA, evidence such as the predisposition of certain MHC class II alleles and the large synovial T cell infiltrate suggests that the pathogenesis of the human disease involves antigen recognition by T cells, particularly in the initial phase of disease development, and probably in disease flare-ups as well [36, 62, 63]. In addition, the efficacy of immunosuppressants, and more recently of targeted anti-T cell therapies such as the anti-costimulatory CTLA4-Ig, supports this concept [64].

The appearance of lesions in rat joints following the introduction of bacterially derived antigens implies that there is a cross-reactive, joint-localized tissue antigen. It has been recently determined the pathogenic immunizing antigen of CFA is the

mycobacterial heat shock protein mHsp65 [65], suggesting a possible case of antigenic mimicry. Hsps are molecular chaperone proteins that are highly conserved among species. Hsp60 is the closest related mammalian hsp to mHsp65, and is present in normal and RA synovium [6, 66]. Interestingly, Hsp60 (or any Hsp) have not been shown to be the direct antigen(s) implicated in AA pathogenesis. However, careful characterization of the mHsp65 protein has identified the existence of a pathogenic epitope, cross-reactive with endogenous cartilage link protein of a cartilage proteoglycan [67]. In addition, there is a distinct regulatory epitope that can actually provide protection from disease and is cross-reactive with endogenous Hsp60. Furthermore, it is possible to induce a protective immune response with mHsp65 protein that is effective in not only against AA, but also SCW and pristane-induced arthritis (the latter model not involving bacterial products) [68–71]. The synovium of animals immunized with mHsp65 contains T cells reactive against Mhsp65/Hsp60 that include both effector and regulatory subtypes [6, 66, 72]. In the monoarticular SCW 100P model, the antigen is introduced directly into the joint, and actually remains at the site for a prolonged period afterwards [73]. After an initial acute episode, swelling subsides virtually completely. However, subsequent systemic administration of SCW 100P fraction, LPS, or superantigen can reactivate the arthritis [3]. T cells isolated from SCW 100P arthritic rats are cross-reactive, recognizing not only SCW, but also mHsp65 [61]. The ability of a variety of bacterial products, including LPS and the products of intestinal overgrowth, to reactivate the arthritic process suggests a possible link to the mechanism of RA disease flare activity [3, 29, 74, 75].

In CIA, the specific targeting of the joint in the subsequent inflammatory response is relatively straightforward. T cells capable of recognizing the collagen molecule, coupled with an appropriately susceptible genetic background, result in the production of both reactive cells and antibodies against autologous type II collagen in articular cartilage [3, 7, 8, 55]. Of the rat models, a significant humoral component has been attributed only to CIA; high titers of anti-collagen type II IgG antibodies are detected, and disease can be transferred to naïve animals using serum from arthritic rats [76, 77]. This autoantibody induction is one of the reasons this model may be favored over the others. In human RA, autoantibodies are a prominent feature of the disease; however, anti-collagen type II antibodies are only present in about 30% of patients, whereas the characteristic rheumatoid factor (RF), an anti-Fcγ autoantibody, is present in 70–80% of patients [78]. RA patients may also have autoantibodies to cyclic citrullinated peptides (CCP) and to cartilage antigens derived from collagen and aggrecan [79]. A pathogenic role for the humoral immune response in RA is further supported by the efficacy of the B cell-depleting anti-CD20 antibody [80, 81]. Rodent models are not characterized by either RF or anti-CCP antibodies [82]. Additionally, despite high circulating anti-collagen antibody levels in CIA, there is no evidence for local (synovial) antibody production as in RA [83, 84]. We and others have observed that, although foci of B cells can be

seen in arthritic rat synovium, true lymphoid follicles such as those found in RA synovium are not present [2, 48].

Despite different means of induction, the developmental sequence of lesions in the joints of the rat arthritis models is quite similar. As early as 72 h after antigen introduction, T cells appear in the perivascular space in the synovium [8, 31, 48, 85]. Subsequently there is fibrin deposition in joint spaces, synoviocyte proliferation, and the appearance of increasing, and eventually very large and predominating, numbers of myeloid lineage cells, particularly neutrophils. Neutrophils are a prominent feature of rat arthritis models, and are the most numerous inflammatory cell in both tissue and synovial fluid [2, 86, 87]. These cells represent a significant source of cytokines, oxygen metabolites, and proteases with the potential to perpetuate joint destruction. Depletion of neutrophils has a significant therapeutic effect on established rat arthritis [59, 87]. Although numerous in human RA synovial fluid, neutrophils are not a conspicuous component of the RA synovium. Other myeloid cells, such as activated macrophages, are the major source of TNF-α and IL-1β. Increased levels of these cytokines has been documented in both RA and the rat models [3, 33, 36, 88, 89]. Besides their pro-inflammatory activities, these cytokines can potentiate both cartilage and bone damage. TNF-α can drive the maturation of osteoclast precursors, and both TNF-α and IL-1β can increase the resorptive ability of mature osteoclasts [90, 91]. In addition, IL-1β particularly induces chondrocytes and synoviocytes to produce matrix-degrading metalloproteases. Both cytokines decrease synthesis of normal cartilage components [92–96]. Anti-TNF and anti-IL-1β-directed therapies have a positive effect in the rat models [2, 40], and are currently used successfully in the clinic for RA [33–35].

In both human RA and rat arthritis models, initial synovial cell hyperplasia transitions into the development of invasive pannus tissue. Pannus formation and the progression of significant cartilage and bone lesions are later features of the arthritic disease process. The relatively late appearance of lesions in the hard tissues has contributed to the idea that bone and cartilage destruction were sequelae of the joint inflammatory process. Some recent evidence from RA clinical trials suggests that this may not be entirely correct. In patients, although anti-inflammatory therapy was shown to reduce clinical signs referable to inflammation, the progression of joint destruction was unchecked [97–99]. Furthermore, in trials involving anti-TNF therapies, synovitis and/or clinical response was unimproved; however, bone erosion was reduced [100, 101]. Rats and humans treated with IL-1R antagonist demonstrated greater protection against bone erosion than joint inflammation [18, 102]. These data suggest that the processes underlying joint inflammation and bone destruction may be mechanistically distinct, at least to some degree. In general, the bone alterations seen in the rat models develop rapidly and are severe compared to human RA. There is a notable periosteal bone formation component as well as bone resorption by osteoclasts. Increased osteoclast precursors are found in both the synovium and synovial fluid in both RA and the rat models [49, 103, 104].

Table 2 - Comparing rat models of arthritis (AA, CIA and monoarticular SCW) with human RA

Disease characteristic	Rat models	Human RA
Antigen	Bacterial (AA, SCW) Bovine collagen type II (CIA)	?
Sex predisposition	None (AA) Female (CIA, SCW)	Female
Acute phase proteins	++	++
Polyarthritis	+ (AA, CIA)	+
Synovitis	++	++
Bursitis/tendonitis	++	++
Cartilage involvement	++ (targeted in CIA)	++
Bone involvement	Early and aggressive (+++ AA; ++ CIA; +/– SCW)	++
Rheumatoid factor	–	+** **70–80% of patients
Neutrophil influx	++ (tissue, synovial fluid)	++ (synovial fluid)
$CD4^+$ lymphocyte influx	++	++
Macrophage in soft tissue	++	++
B cells/Plasma cells	Random lymphocytic infiltrates (CIA); Role of B cells unclear (AA, SCW)	Synovial lymphoid aggregrates; Local antibody production
Elevated synovial cytokines:	++	++
TNF-α	+	+
IL-1β	+	+
IL-6	+	+
Edema	++	++
Disease course	~3 weeks subsides into fibrosis/ ankylosis eventually	Frequently decades
Disease flares	– (AA, CIA) + (SCW)	+

In summary, many of the cell types involved and the molecular mechanisms implicated in human RA and the rat models of arthritis are identical. Most obviously, the prolonged time frame, and the recurrent nature of disease exacerbations of human RA are different (although recurrence is reported to occur in SCW), as are some other features of disease, including presence of RF and synovial lymphoid follicles. The preventative and disease-modifying therapeutic effects of a variety of non-steroidal anti-inflammatory drugs suggests a greater dependence on prostaglandins for disease development and maintenance of the rat models [1, 4, 105]. No such activity has been seen in RA patients; however, it is likely that none are treated as near to the onset of their disease. Table 2 highlights the key features of the rat model

pathology as compared with RA. Between the three models, which we run in female Lewis rats, we find that differences are primarily quantitative, and the general features of the disease are very similar overall. These are reviewed in the next section.

Disease parameters

The onset of ankle swelling is monitored to indicate the development of arthritic disease following administration of an inducing agent. After the injection of CFA, there is a significant increase in the ankle volume of the injected group compared to controls. Paw swelling in AA is robust, with increases up to 3.5-fold times control volume. In general, maximal swelling occurs by day 19 and then plateaus. Our studies are generally completed at this point. If the study is continued out to 40–50 days, the paw volume may come down somewhat, but remains significantly elevated compared to non-arthritic controls. Paw swelling in CIA is less severe, generally reaching only about a 2-fold increase over the control volume. In onset, swelling generally occurs slightly later than in AA, beginning around day 14 after the first injection of bovine type II collagen. The greatest paw volume is seen at about day 21, and also plateaus in severity. The least severe paw swelling occurs in the SCW model, with affected paws only increasing in volume by 1.5-fold. In this model, there is a small initial paw swelling observed immediately following the intra-articular (i.a.) injection of SCW 100P, but this rapidly subsides by days 3–4. After the second exposure to antigen by intravenous (i.v.) challenge 2–3 weeks later, there is a rapid and predictable reappearance of paw swelling of the magnitude described. This swelling is maximal at 2–3 days after i.v. SCW and also plateaus. Due to the synchronization and predictability of the response, this model is favored as a model of arthritic flare, and is being used more commonly to evaluate novel therapies. A detailed description of each of these models is provided in a later section, including examples of paw swelling.

Clinical pathology

The systemic manifestation of the inflammatory response to the inciting agents used in animal models of arthritis is reflected in several peripheral blood-based parameters. Normal female Lewis rats used in our studies typically have total leukocyte counts around 9×10^9–10×10^9 cells/L, 80% of which are lymphocytes. Neutrophil counts are usually low, not exceeding 2×10^9 cells/L (Tab. 3). In the AA model, within the 1st week after CFA administration prior to the development of contralateral ankle swelling, neutrophil counts increase dramatically, about 5–7-fold normal. These high counts persist throughout the typical study period (19 days). Concomitantly, there is an increase in serum fibrinogen levels, from normal levels (roughly

Table 3 - Comparison of various blood parameters in rat models of arthritis

Parameter	Additional parameters for assessing inflammation and tissue destruction in rat arthritis models			
	Control value, mean ± SD	Day 19 AA, mean ± SD	Day 22 CIA, mean ± SD	Day 24 SCW (100P), mean ± SD
Clinical pathology				
Total leukocyte count, $\times 10^9$/L	9.1 ± 1.4	17.5 ± 2.2	11.5 ± 1.5	9.5 ± 1.3
Total neutrophils, $\times 10^9$/L	1.2 ± 0.6	8.8 ± 1.3	4.8 ± 1.4	1.2 ± 0.3
Total lymphocytes, $\times 10^9$/L	7.4 ± 0.7	7.2 ± 0.9	6.0 ± 0.8	7.8 ± 1.0
Blood fibrinogen, mg/dL	213 ± 15	806 ± 40	436 ± 25	438 ± 150
Acute phase proteins				
α1 acid glycoprotein, μg/mL	175 ± 90	1845 ± 200	490 ± 150	440 ± 100
Haptoglobin, mg/mL	0.5 ± 0.1	3.9 ± 0.9	1.4 ± 0.3	1.5 ± 0.3
Soluble bone & cartilage markers				
Collagen type I telopeptides (RatLaps) ng/mL	35 ± 9	82 ± 20	57 ± 15	ND
Collagen oligomeric matrix protein (COMP), μg/mL	1.4 ± 0.2	4.7 ± 0.5	3.3 ± 0.5	ND
Quantifiable μCT-derived bone parameters				
Bone volume (arbitrary units)	35 ± 1	21 ± 8	29 ± 2	ND
Bone roughness (arbitrary units)	1640 ± 250	ND	10950 ± 3840	ND

ND, not done.

200 mg/dL) to 4-fold increase (800 mg/dL). An elevated erythrocyte sedimentation rate (ESR) has also been documented in AA, from day 4 and peaking at day 12 but remaining high up to day 50 [89]. In CIA, the maximal neutrophil increase is less (3–4-fold), as is the fibrinogen increase (400 mg/dL). No increase in neutrophils is detected in monoarticular SCW model. However, fibrinogen levels increase to approximately the same levels as those seen in CIA (400 mg/dL). Typical values for each model are shown in Table 3.

Acute-phase proteins

The acute inflammatory nature of all three models is reflected in the increases in serum levels of acute-phase proteins (APPs). We have typically used alpha-1-acid gly-

coprotein and haptoglobin as disease biomarkers in our rat models. CRP, the APP most commonly followed in human RA patients, is not a major induced APP in the rat, and is therefore not as useful as it is in humans. Similar to the other parameters we have examined, the trend for the greatest magnitude of increase in AA is repeated. We have observed roughly 10-fold increased levels of alpha-1-acid glycoprotein and 7–8-fold increase in haptoglobin levels. CIA and monoarticular SCW produce similar elevations in both alpha-1-acid glycoprotein and haptoglobin, roughly 3-fold increases in both parameters. Representative values are shown in Table 3.

Gene expression

We and others [30, 106] have profiled the expression of a number of genes in the joint during the development and establishment of rat arthritis. By semi-quantitative PCR, Schmidt-Weber et al. [106] detected increased mRNA expression for IFN-γ, IL-1β, IL-5, IL-6, TNF-α and IL-10 in the draining (popliteal) lymph node in AA. Most of these genes peaked in expression on day 6, before the onset of clinical arthritis. In the affected synovial membrane, peak IL-6 was found on day 16 and peak IL-1β occurred from days 13 to 20. Interestingly, these researchers reported that no TNF-α mRNA was detected in the dissected synovial membrane. Another approach, including our own, has been to use the entire affected ankle for RNA preparation. The mRNAs we have examined in the paws reflect multiple parameters associated with arthritis, including markers of cellular infiltration [CD4, B29 (B cell), CD11b], NF-κB-induced inflammatory cytokines and related molecules (IL-1β, TNF-α, IL-6, iNOS, COX-2), matrix metalloproteases (MMP3, MMP13), anti-inflammatory cytokines (IL-10, TGF-β), and bone-associated markers (TRAP, RANKL, cathepsin K). In our quantitative PCR studies, the earliest mRNAs to achieve earliest statistically significant elevation in AA joints at day 12 were CD11b, MMP3, IL-10. In our hands we were also able to consistently detect TNF-α in these whole joint preparations. The majority of genes examined achieved peak mRNA expression at day 16 and remained significantly elevated at day 19. This contrasts with paw swelling, which often is still increasing on day 16 and peaks at day 19. Interestingly, a very similar pattern of gene expression has been seen in CIA, although the increases generally occur 2–3 days later than in AA. Of the genes examined in all three models, we have primarily observed a difference in the expression level. The magnitude of mRNA increase was less in CIA than AA for a number of genes, including CD11b, TNF-α, IL-1β, iNOS, MMP13, and TRAP. The magnitude of increase is least in monoarticular SCW, although there is a marked induction of IL-6 at 24 days (3 days after reactivation); greater than CIA or AA at their respective final study day. Table 4 summarizes the relative magnitude of mRNA increase compared to housekeeping gene GAPDH on the final day of each model. Not all of the genes we have analyzed demonstrated regulation: for example, IL-4,

Table 4 – Comparison of joint gene expression in rat models of arthritis

	Joint mRNA expression: fold increase over baseline		
Gene	**AA**	**CIA**	**SCW (100P)**
Cell marker			
CD11b (myeloid)	10–25×	5–20×	6–10×
CD4 (T helper)	2–4×	2–3×	2×
Pro-inflammatory			
TNF-α	5–15×	3–10×	4–5×
IL-1β	10–100×	10–60×	15×
IL-6*	250–3000×*	500–6000×	>6000×
COX-2	5–25×	2–6×	6×
iNOS	30–150×	15–70×	7×
Anti-inflammatory			
IL-10	2–3×	2–3×	ND
TGF-β	2–5×	2–3×	ND
Metalloprotease			
MMP3	15–50×	15–40×	40×
MMP13	15–40×	3–20×	15×
Osteoclast-associated			
RANKL	50×	20×	6×
TRAP	5–10×	2–5×	5×
Cathepsin K	20×	10×	ND

**Higher baseline associated with mineral oil injected control group (AA baseline) compared to saline or IFA injected controls (SCW, CIA baseline)*

COX-1, and B29 (Ig beta) failed to demonstrate significant change in expression or differential expression between the experimental groups in all models.

Cytokine levels

Plasma/serum and tissue cytokine levels have been studied by several investigators, using a variety of bioassay and ELISA methods. In AA, Philippe et al. [5] noted a spike in serum levels of TNF-α and IL-6 (not IL-1β, which remained unchanged) between 6 and 12 h after CFA injection. These levels returned to baseline and then gradually increased up to day 20, with a greater magnitude of increase observed for IL-6 than TNF-α. Szekanecz et al. [89] reported concomitant increases in serum and joint cytokine levels between days 11 and 25 for TNF-α and IL-6, and increased