Utility of animal models for identification of potential therapeutics for rheumatoid arthritis

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ABSTRACT

Animal models of rheumatoid arthritis (RA) are widely used for testing potential new therapies for RA. However, the question of which animal model is most predictive of therapeutic efficacy in human RA commonly arises in data evaluation. A retrospective review of the animal models used to evaluate approved, pending RA therapies, and compounds that were discontinued during phase II or III clinical trials found that the three most commonly used models were adjuvant-induced arthritis (AIA) in rats and collagen-induced arthritis (CIA) in rats and mice. Limited data were found for more recently developed genetically modified animal models. Examination of the efficacy of various compounds in these animal models revealed that a compound's therapeutic efficacy, rather than prophylactic efficacy, in AIA and CIA models was more predictive of clinical efficacy in human RA than data from either model alone.

Rheumatoid arthritis (RA) is a chronic, inflammatory, systemic autoimmune disease that affects about 1% of the general population in Western countries and is two to three times more common in women than in men.[1] Although the aetiology and pathogenesis of RA is not yet fully understood, the disease is characterised by aggressive synovial hyperplasia (pannus formation) and inflammation (synovitis), which, if left untreated, lead to progressive destruction of joint cartilage and bone. The destructive lesions result from immune responses and non-antigen-specific innate inflammatory processes.[2]

Studies of synovial tissue taken during different phases of the disease have increased our understanding of the mechanisms involved in joint destruction and response to treatment.[3] In addition, efficacy of specific therapeutic reagents gives us a greater understanding of the disease process in RA. For example, the efficacy of abatacept, which blocks activation of T cells through the CD28 costimulatory receptor is evidence for the pathogenic role of T cells in RA.[4] Blocking proinflammatory cytokines such as tumour necrosis factor (TNF)α, interleukin (IL)1 and IL6 has led to improvement in disease scores in patients with RA. A critical role for B cells has been validated by the recent clinical success of B cell depleting agents for the treatment of RA.[5 6] Activated synoviocytes produce many key cytokines and mediators that may contribute to the inflammation and joint destruction associated with RA as illustrated in fig 1. Many therapies designed to inhibit these cytokines and mediators are currently in clinical trials for RA (tables 1–3). Current approaches to drug therapy for RA include non-steroidal anti-inflammatory drugs (NSAIDs) for pain treatment, disease-modifying antirheumatic drugs (DMARDs) and newer biological agents that target specific proinflammatory cytokines, cell surface receptors or various cell types (table 4).[1]

Animal models of arthritis have been used to provide insight into the underlying disease process to identify new targets for drug therapy, and to identify potential new therapeutic agents for RA.[7 8] These animal models share features with human RA,[9] but they also have differences.[8] It is important to select an animal model that has similar pathology and/or pathogenesis to human RA and that has the capacity to predict efficacy of a given therapeutic agent in humans. Additionally, it is desirable to have a model that is reproducible in mechanism and outcome. Wherever possible, the target should be validated in the animal model and human disease. The objective of this retrospective review article was to determine whether evaluation in widely used animal models could predict a compound's efficacy, or lack of efficacy, in human RA. To achieve this, we identified those animal models used to evaluate the efficacy of approved and pending RA therapies. We also discuss additional animal models of RA that can be used to evaluate potential therapeutics. These data can be used to establish guidelines for the use of these animal models in the preclinical development of RA therapies.

METHODS

For this review, we searched databases of publicly available information from 2000 to 2007 (most recent search was in January 2007) to identify animal models of arthritis and determine which models were most commonly used. There were only a few reports of compounds tested in models other than adjuvant-induced arthritis (AIA) and collagen-induced arthritis (CIA); therefore, we focused our subsequent searches for information in these animal models. We focused on compounds either on the market, or in phase II or III of clinical development since phase I trials are usually designed to evaluate safety and not clinical efficacy. Subsequently, the literature was searched by each compound name, including generic names and numbers, and crossreferenced with the various animal models. The data were then examined to try to determine whether efficacy in human RA could be predicted by the animal model and treatment protocol chosen. Compounds that failed in phase II or phase III clinical trials were also included to determine whether efficacy in any particular model had a higher failure rate. However, the reasons for clinical failure may be due to toxicity or other issues unrelated to efficacy, and this information is often not disclosed. Several

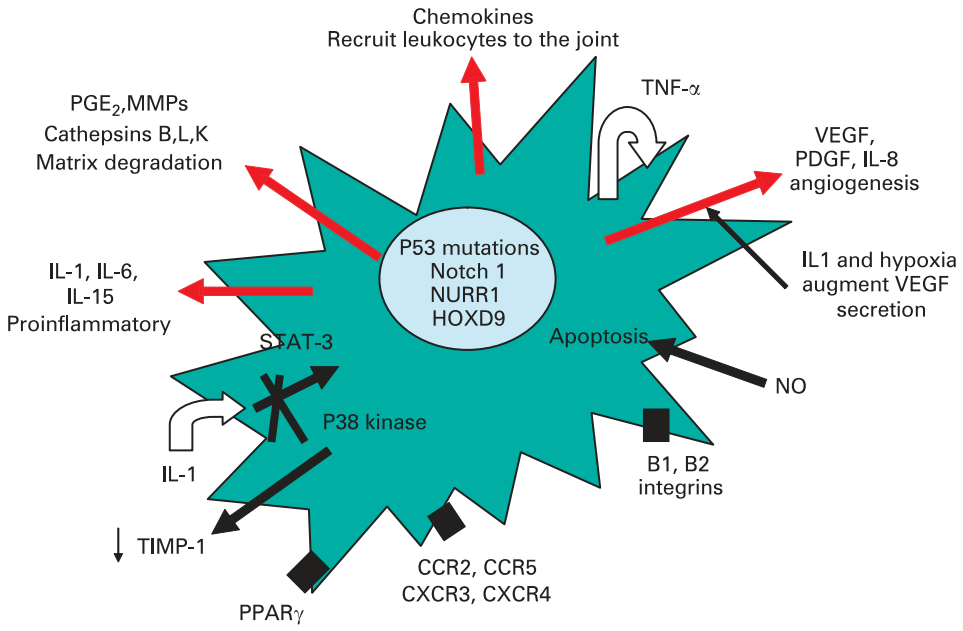

Figure 1 Schematic of a synoviocyte indicating potential therapeutic targets, including many that are targeted by drugs currently in clinical trials or on the market.

compounds that are in clinical trials for RA were not tested in the AIA or CIA models, but may have been tested in other models of arthritis due to species-specificity or pharmacokinetic issues. Other compounds have moved into RA clinical trials based on clinical success in other areas, such as hydrochloroquine, which is an antimalarial drug. Genetically modified animals have also provided proof-of-concept for other compounds to move forward in development. Table 1 summarises compounds in phase II or phase III clinical trials for RA that have moved forward without data in AIA or CIA models.[10–19]

COMMONLY USED ANIMAL MODELS

Numerous animal models of arthritis exist, many of which have been used to evaluate compounds that may be potential new therapies for RA. We identified that the three most commonly used models of RA for the testing of potential therapeutic agents are AIA in rats, CIA in rats and CIA in mice. Although newer models exist, sufficient data linking preclinical efficacy with proven clinical efficacy in RA is not typically available in these models. Thus, we limited our extensive clinical data collection to the AIA and CIA models.

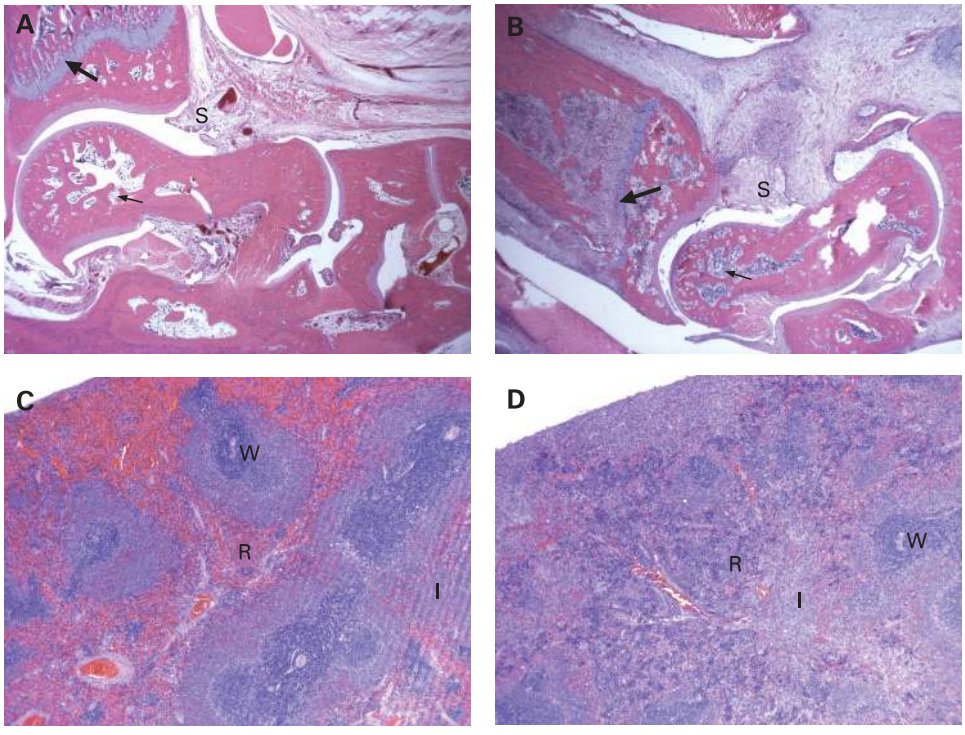

Figure 2 Histology of ankle joints and spleens from control Lewis rats and rats with adjuvant-induced arthritis (AIA). Normal synovium (s), normal distal growth plate (large arrow) and normal tarsals (small arrow) are represented in panel A. An ankle from an arthritic rat demonstrating marked bone resorption in the distal tibia (large arrow) and minimal resorption in small tarsals (small arrow) in association with severe synovitis and periarticular inflammation (B). Magnification = 16×. The spleen from a normal rat with normal white (w) and red (R) pulp is shown in (C). The spleen from an arthritic rat shown in (D) has moderate pyogranulomatous inflammation (I), moderate lymphoid atrophy (W) and marked extramedullary haematopoiesis (EMH) in red pulp (R). Magnification = 50×. (Photomicrographs provided by Bolder BioPATH, Inc., Boulder, Colorado, USA.)

Table 1 Compounds progressed in clinical development without preclinical data in adjuvant-induced arthritis (AIA) or collagen-induced arthritis (CIA)

Compound	Mode of action	Clinical phase	In vivo validation	Reference
Rituximab	Anti-CD20	Approved	B cell depletion in non-human primates	Looney[10]
Infliximab	Anti-TNF	Approved	Effects on Inflammatory processes	Maini and Feldman[11]
Adulimimab (D2E7)	Anti-TNF	Approved	None found on this particular compound	
Hydrochloroquine	Antimalarial	Approved	None found on this particular compound	
Belimumab	Anti-Blys	III	B cell depletion in non-human primates	Halpern *et al*[12]
TRU-015	Anti-CD20	II	B cell depletion in non-human primates	Trubion Pharmaceuticals[13]
HuMax-CD20	Anti-CD20	II	B cell depletion in laboratory tests and animal studies	Genmab[14]
Cimzia	F(ab′)PEGylated anti-TNF	III	None found on this particular compound	
AVE9897 (MLN 3897)	CCR1 antagonist	II	None found on this particular compound, however, CCR1 antagonism works therapeutically in mouse CIA	Amat *et al*[15]
Rhu-Dex	Anti-CD80	II	None found on this particular compound	
CRx-102	Dipyridimole and prednisolone	II	None found on this particular compound	
681323 (GSK)	p38 inhibitor	II	None found on this particular compound	GlaxoSmithKline[16]
856553 (GSK)	p38 inhibitor	II	None found on this particular compound	GlaxoSmithKline[16]
247150	iNOS inhibition	II	None found on this particular compound	
Rosiglitazone XR (GSK)	PPAR agonist	II	None found on this particular compound, however, PPAR agonism works prophylactically in mouse CIA	Tomita *et al*[17]
RJW 445380	Cathepsin S inhibitor	II	Decreased CIA in cathepsin S deficient mice	Nakagawa *et al*[18]
UK 427857	CCR5 inhibitor	II	None found on this particular compound, however, CCR5 antagonism works prophylactically in mouse CIA	Yang *et al*[19]

CCR, Chemokine (C-C motif) receptor; iNOS, inducible nitric oxide synthase; PEG, polyethylene glycol; PPAR, peroxisome proliferator-activated receptor; TNF, tumour necrosis factor.

Rat AIA

AIA in rats was the first animal model of RA to be described,[20] and it is still widely used in the preclinical testing of new agents for arthritis, especially NSAIDs. Classic AIA is induced in Lewis rats by a single intradermal injection of complete Freund adjuvant (CFA).[7] The AIA model is characterised by reliable, rapid onset and progression of a robust and easily measurable polyarticular inflammation, marked bone resorption and periosteal bone proliferation.[7] Severe polyarthritis develops rapidly – clinical signs of arthritis usually appear about 10 days after

Table 2 Efficacy (therapeutic or prophylactic) in animal models of candidate rheumatoid arthritis (RA) drugs currently in phase II and III trials

		Rat AIA		Rat CIA		Mouse CIA		
Mechanism of action	Example	Prophylactic	Therapeutic	Prophylactic	Therapeutic	Prophylactic	Therapeutic	References
Phase III:								
Dual COX/LT inhibitor	Licofelone (ML-3000)		+					Laufer *et al*, Gay *et al*[93 94]
IL1 and IL6 synthesis inhibitor	TA-383	++	++			+		Ueno *et al*[95]
Phase II:								
P38 MAP kinase inhibitor	Scio-469				+			Brahn *et al*[96]
B cell activation antagonist	Atacicept (TACI-Ig)						+	Gross *et at*[97]
Calcineurin inhibitor	ISAtx-247						+	Isotechnika[98]
Inhibits production of cytokines (IL1β, TNFα, IL6)	K-832			++	++	++	++	
Antibody to IL15	AMG 714 (HuMax-IL15)					+		
IL1 receptor antagonist	AMG 719				+			
IL12, IL23 inhibitor	Apilimod mesylate (STA-5326)	++	++	++	++			
Adenosine A3 receptor agonist	CF-101	+				+		Baharav *et al*[99]
Oestrogen receptor-β agonist	Prinaberel (ERB-041)		+					Steffan *et al*, Harris *et al*[100 101]
COX inhibitor, NO donor	Nitronaproxen		+					Cicala *et al*[102]
PGE_2 antagonist	SMP-114					++	++	
5-lipoxygenase (LTB_4 antagonist)	CP-195543					+ (IL1 model)		Showell *et al*[103]
Syk kinase inhibitor	R788		+		+			
JAK 3 inhibitor	CP-690,550		+		+			Milici *et al*[104]
Anti-RANKL	Denosumab			−				Kamjo *et al*[105]
CCR2 antagonist	INCB3284	+						Brodmerkel *et al*[106]

+, Efficacy; −, no effect . Where there is a ++ sign covering prophylactic and therapeutic efficacy, efficacy was demonstrated but it was not possible to determine whether it was prophylactic or therapeutic efficacy from the information available.
AIA, adjuvant-induced arthritis; CIA, collagen-induced arthritis; CCR, chemokine (C-C motif) receptor; COX, cyclo-oxygenase; IL, interleukin; JAK, Janus kinase; LT, leukotriene; LTB, lymphotoxin beta; MAP, mitogen activated protein; NO, nitric oxide; PGE_2, prostaglandin E_2; RANKL, receptor activator for nuclear factor κB ligand; Syk, spleen tyrosine kinase; TNF, tumour necrosis factor.

adjuvant injection – but rarely lasts longer than a month.[21] Histology of a paw taken from a rat with AIA on day 14 post immunisation is shown in fig 2. The cell infiltration, particularly neutrophils, and the joint destruction are evident.

AIA is a T cell-dependent disease that shares some features with human RA, including swelling of the extremities, cartilage degradation, loss of joint function and lymphocyte infiltration of the joints. Bone resorption is prominent in AIA; damage to cartilage occurs to a lesser degree than in rat CIA and human RA.[22] Unlike human RA, the spine, the gastrointestinal and genitourinary tracts, the skin and the eyes[9 21] are also affected, similar to human spondyloarthropathies. The AIA model is T cell and neutrophil dependent, and complement-independent. There is also no documented role for B cells.[22–26] In addition, T helper (Th)1 and Th17 inflammatory cytokines have been associated with AIA. Increased levels of TNF, interferon γ (INFγ), IL1, IL6 and IL17A mRNA have been detected in lymph nodes and/or inflamed joints of rats with AIA.[27 28] Blockade of TNF, IL1, IL21 and IL17A in rats with AIA ameliorates the disease, indicating that these cytokines contribute to the pathology in this model.[29–31] For a more detailed review of the AIA model, see van Eden and Waksman.[32]

Rat CIA

CIA was first described in rats[33] and is a commonly used model for assessing the efficacy of potential new therapeutic agents for RA. Severe polyarthritis is induced in rats by intradermal/subcutaneous injections of homologous or heterologous type II collagen emulsified in IFA.[33] It is characterised by marked cartilage destruction associated with immune complex deposition on articular surfaces, bone resorption and periosteal proliferation, together with synovitis and periarticular inflammation as shown in fig 3.[7] The robust immune response involves CII-specific T cells and B cells; the latter produce antibodies to type II collagen.[34]

Rat CIA has many similarities to human RA.[9 35] As in human RA, females are more susceptible. The onset of arthritis is rapid, typically developing 10–13 days after immunisation, peaking at about day 20 and then gradually declining.[7 36] Rat CIA differs from human RA in that it is self-limiting and not characterised by exacerbations and remissions. In addition, the inflammatory cell infiltrate in rat CIA consists predominantly of polymorphonuclear cells, whereas a high proportion of mononuclear cells are seen in human RA.[37] The rat CIA model differs from the AIA model in several ways: arthritic disease is less common and less severe,[38] there is greater involvement of B cells,[39] and the CIA model is complement dependent.[24]

Mouse CIA

CIA can be induced by immunisation of genetically susceptible strains of mice with heterologous type II collagen in CFA.[40] Following immunisation, the animals develop an autoimmune polyarthritis that is characterised by severe cartilage and bone erosions. The lesions in affected joints are similar to those seen in rat CIA.[7] Mouse CIA shares several clinical, histopathological and immunological features with human RA: clinical features include erythaema and oedema; histopathological features include synovitis, pannus formation and cartilage and bone erosion as shown in fig 4. Immunological features include high levels of antibody to type II collagen, production of rheumatoid factors[41] and hyper γ-globulinaemia.[42] Typically, mouse CIA is characterised by symmetrical joint involvement with the peripheral joints affected.[9] The cell infiltration into the joint space, synovial hyperplasia and marginal erosions are similar to those observed in AIA and in human RA. By contrast, periostitis is present in CIA, but not in human RA.[43] Susceptibility to CIA and RA is strongly associated with the expression of specific major histocompatability complex (MHC) class II molecules,[37] specifically, I-Aq and I-Ar in the mouse, and human leukocyte antigen (HLA)-DR1 and DR4 in the human.[43–45]

Studies of CIA in mice have indicated that autoantibodies, inflammatory cytokines and multiple cell types including T cells, although T cells play a role in the pathogenesis of CIA.[40] As in human RA, several pro and anti-inflammatory cytokines are expressed in the joints of mice with CIA, including TNFα and IL1β, IL6, IL1Ra, IL10 and transforming growth factor (TGF)β.[46] IL12 and IL23 also appear to be involved in the pathogenesis of mouse CIA.[47 48]

The mouse CIA model has a slower onset and a more prolonged duration than the rat CIA model.[49] Moreover, differences exist in the immune response between the two species.[50] The mouse has the advantage that there are extensive immunological and genetic tools available to manipulate the disease in this species.[51] There are several different protocols for inducing CIA in mice. Typically, the mice are immunised with bovine or chick type II collagen is emulsified in CFA followed by a boost of collagen approximately 3 weeks later.[52] In some cases, the mice are given lipopolysaccharide (LPS) around the time of the boost to induce the disease to occur more rapidly with less variability in onset.[53] We found that most compounds discussed in this review were tested in CIA models induced with heterologous cartilage and without an LPS boost. However, all data are included, regardless of the CIA model used.

Treatment regimens

In this review, we considered treatment regimens to be prophylactic when dosing with the study drug was started at or before immunisation, or before disease onset. When dosing with study drug was started only after clinical signs of disease were observed, we considered the treatment regimen to be therapeutic.

COMPOUNDS APPROVED AND IN DEVELOPMENT

We identified several therapies approved for use in the US, Europe and Japan from the initial database search. Table 4 summarises these approved therapies by their therapeutic and/or prophylactic efficacy in the three most commonly used animal models.[22 36–38 51 54–92] DMARDs such as methotrexate, ciclosporine, gold compounds and penicillamine have been tested in all three animal models, generally for prophylactic efficacy rather than therapeutic efficacy. However, the majority of these DMARDs did not follow a classical drug development pathway. These drugs were being used in other disease areas (eg, oncology for methotrexate) or they had anecdotal efficacy. Penicillamine has been used to treat arthritis for over 50 years, whereas gold compounds have been used to treat arthritis for more than 75 years. Thus, these compounds were not rigorously tested for efficacy in vivo prior to use in the clinic. The in vivo data that are available was typically generated after these compounds were being used as therapeutics for RA. The AIA model was commonly used to evaluate corticosteroids, NSAIDs and selective cyclo-oxygenase-2 (COX-2) inhibitors; these classes of agent showed therapeutic efficacy in this model. The NSAID indomethacin also showed therapeutic efficacy when tested in the rat and mouse CIA models.[36 62 65 70–72] It is important to note that NSAIDs are effective in treating the pain

Table 3 Efficacy (therapeutic or prophylactic) in animal models of rheumatoid arthritis (RA) of drugs suspended from phase II or III clinical development as treatments for RA

		Rat AIA		Rat CIA		Mouse CIA		
Mechanism	**Example**	**Prophylactic**	**Therapeutic**	**Prophylactic**	**Therapeutic**	**Prophylactic**	**Therapeutic**	**References**
Phase III:								
TACE inhibitor	DPC 333 (BMS-561392)				+	+		Lorenz *et al*[107]
Anti-cytokine, anti-T cell	Esonarimod	++	++					Noguchi *et al*[108]
NSAID	Amiprilose	++		++	++			Kieval *et al*[109]
Increase T suppressor cells	Nuclomedone			+	−			Komoriya *et al*[110]
PGE synthesis inhibitor	FK 3311	+		+				Harris *et al*, Tsuji *et al*[101 111]
MMP inhibitor	Cipemastat (Trocade)	−	–	−				Ishikawa *et al*[112]
Phase II								
Th2 induction, NFκB inhibition	IL11		+		+		+	Albert *et al*, Walmsley *et al*[113 114]
Decrease IL1 release	TOK-8801	+		+		++	++	
VLA-4 antagonist	ZD-7349					+		
Dual COX/LT inhibitor	Tepoxalin		+					Argentieri *et al*[74]
Complement inhibitor (humanised anti-C5) monoclonal antibody)	Eculizumab (5G1.1)						+	Linton and Morgan, Wang *et al*[24 115]
Antibody to IL12p40	IL12 mAb (ABT 874)						+	
O_2 radical scavenger	Superoxide dismutase	++	++					Shingu *et al*[116]
P38 MAP kinase inhibitor	Doramapimod (BIRB 796)						+	Nabozny *et al*[117]
P38 MAP kinase inhibitor	TAK 715	+						Miwatashi *et al*[118]
Bisphosphonate, IL1 antagonist	TRK 530	+				+		Tanahashi *et al*, Takaoka *et al*[119–121]
ICE inhibitor	Pralnacasan (VX740)						+	Linton, Ku *et al*[122 123]
IL4 (B cell stimulatory factor 1)	IL4					+	+	Cottard *et al*, Kim *et al*[124 125]
Oral TNFα inhibitor	AGIX-4207			+				Sundell *et al*[126]
MCP-1 inhibitor	Bindarit		+					Guglielmotti *et al*[127]
Immunomodulator	SM 8849	++	++			+		Nagai *et al*[128]
IFNβ	Rebif						+	van Holten *et al*[129]
Targets α (v) β 3	RGD-4C_D(KLAKLAK)$_2$					+		Gerlag *et al*[130]

+, Efficacy; −, no effect. Where there is a ++ sign covering prophylactic and therapeutic efficacy, efficacy was demonstrated but it was not possible to determine whether it was prophylactic or therapeutic efficacy from the information available.
AIA, adjuvant-induced arthritis; CIA, collagen-induced arthritis; COX, cyclo-oxygenase; ICE, interleukin 1β converting enzyme; IFN, interferon; IL, interleukin; LT, leukotriene; mAb, monoclonal antibody; MAP, mitogen activated protein; MCP, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; NFκB, nuclear factor κB; NO, nitric oxide; NSAID, non-steroidal anti-inflammatory drug; PGE_2, prostaglandin E_2; RA, rheumatoid arthritis; TACE, TNFα converting enzyme; Th, T helper; TNF, tumour necrosis factor; VLA-4, very late antigen-4.

associated with arthritis, but these drugs do not inhibit progression of joint damage, despite demonstrating efficacy in the rodent models of arthritis. In order to identify compounds that will affect disease progression as well as affect signs and symptoms of disease, biomarkers need to be identified in the arthritis models.

Newer RA therapies such as the IL1 receptor antagonist (IL1Ra) anakinra and the TNF antagonist etanercept have shown therapeutic efficacy in all three animal models of RA.[22 42 79–83]

Drugs with various mechanisms of action currently in clinical development for the treatment of RA (ie, in phase II or III clinical trials) have been evaluated for prophylactic and/or therapeutic efficacy in the AIA and rodent CIA models (table 2).[93–106] Many of these newer drugs are targeted to specific aspects of the underlying pathophysiology, and the animal model used usually depends on the mechanism of action of the drug being tested. For example, anti-inflammatory agents tend to be tested in the rat AIA model, whereas the more recent mitogen activated protein (MAP) kinase inhibitors were tested in the rat CIA model.

Taken together, the data from RA drugs that are approved or are progressing in clinical development suggest that therapeutic efficacy in animal models of RA predicts clinical efficacy. Most approved drugs have data on therapeutic efficacy from AIA and CIA models, indicating that data from both types of animal models are more predictive of clinical efficacy than are data from either model alone.

COMPOUNDS DISCONTINUED OR SUSPENDED

Table 3 summarises the efficacy data from animal models of RA for compounds suspended in phase II and III clinical trials.[24 65 107–130] Most of these compounds were targeted towards specific aspects of the pathophysiology of RA. Some of the compounds discontinued during clinical development showed efficacy in either the AIA or CIA models of RA. However, most

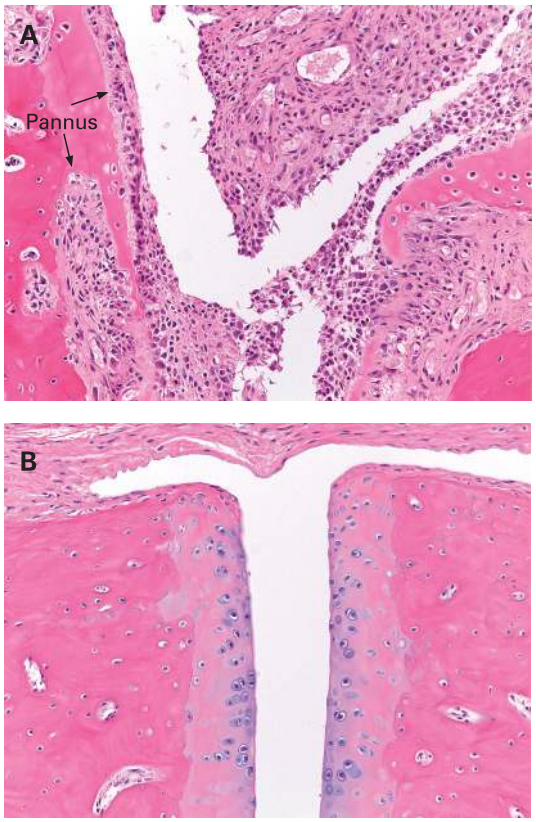

Figure 3 Histology of the ankle joint from a rat with collagen-induced arthritis (CIA) on day 35 (A) after initial immunisation demonstrating the cellular infiltration and cartilage destruction in this model and from a naïve rat (B). Magnification = 20×.

compounds suspended in phase II or III clinical trials did not show therapeutic efficacy in one or more RA animal models.

In addition, there are many compounds that did not demonstrate efficacy in animal models of arthritis and therefore, were not tested in humans. Thus, animal models can be very important to determine lack of efficacy, as well as efficacy. These data are not captured in this review as this review focuses on compounds that were tested in clinical trials.

OTHER AVAILABLE MODELS

Primate model

CIA in the rhesus monkey is a non-predictable and often severe model of human RA.[131] Consequently, the use of the model is often limited to species-specific reagents that can not be tested in lower species. Therapies that have shown efficacy in the primate model include the anti-IL6R antibody, Toclizumab, the humanised monoclonal antibody against the IL2 receptor, Daclizumab, and the chemokine (C-C motif) receptor (CCR)5 antagonist SCH-X.[132–134]

Rabbit model

Antigen-induced arthritis in rabbits, first described by Dumonde and Glynn,[135] has joint pathophysiology similar to RA and is especially important for evaluating intra-articular therapies. This model has been successfully used to test various gene therapy strategies.[136 137]

Rat models

Other rat models of RA include the streptococcal cell wall (SCW) model, and the HLA-B27 transgenic model. The SCW model is induced by a single intraperitoneal injection of streptococcal cell wall fragments. This results in a non-T cell dependent phase followed by a chronic, inflammatory, T cell dependent phase associated with the production of high levels of inflammatory cytokines and erosive cartilage damage in the arthritic joints.[138–140] The spontaneous remissions and exacerbations of chronic inflammation that occur in the joints of the SCW model are similar to those observed in RA.

In the HLA-B27 rat model, rats that are transgenic for HLA-B27 express high levels of human HLA-B27 and β_2 microglobulin proteins.[141] The rats are normal at birth but develop T cell-mediated, spontaneous chronic inflammation of many organ systems as they age, including arthritis, inflammatory bowel disease and psoriasis.[142 143] The disease is similar to human spondyloarthropathies and is dependent on bacterial flora and the immune system.[144] Because the HLA-B27 rat model is relatively new, few compounds for treating RA have been tested in this model.

Mouse models

Several additional models of arthritis in mice have also been developed, including the HLA-DR4 mouse, the K/BxN mouse, the proteoglycan (PG) model, the severe combined immunodeficiency (SCID) model, the TNF transgenic model, the SKG mouse and DNase II–/– interferon (IFN)iR–/– mice. The HLA-DR4 mouse model that was recently described, lacks all endogenous mouse class II genes and expresses the RA susceptibility allele HLA-DRB1*0401.[145] Disease is predominantly in females and there are rheumatoid factors, plus the expression of class II molecules on antigen-presenting cells and T cells, similar to human RA.

The K/BxN mouse model, was first described in 1996.[146] K/BxN mice spontaneously develop a severe, chronic, progressive inflammatory arthritis at about 27 days old without the need for administration of an external antigen. This model is similar to human RA in many respects and involves not only T cells but also B cells, which secrete autoantibodies that promote joint destruction.[147] Moreover, transfer of serum or purified Ig from arthritic K/BxN mice to recipient normal mice induces a rapid and profound erosive synovitis, similar to human RA, that is dependent on neutrophils, mast cells, macrophages and inflammatory mediators.[148–150]

Another very valuable model is the PG (human proteoglycan or aggrecan) model in the mouse. This model is chronic and relapsing and, like the K/BxN model, is also T cell and antibody mediated.[151 152]

In the SCID model, RA synovial tissue or cultured fibroblasts and normal cartilage are co-implanted under the renal capsule of SCID mice.[153–155] Many of the features of the rheumatoid synovium last for at least 12 weeks. As a result, this model can be used to test human specific agents that may be potential therapeutic agents for RA, such as humanised monoclonal antibodies.[156] The SCID mouse model has been used in gene transfer studies to identify potential therapeutic targets for RA, including cytokines, activating factors of synovial cells, matrix-degrading enzymes and regulators of cell survival and apoptosis.[157 158]

Chronic inflammatory polyarthritis also develops in mice expressing 3′ modified human TNF transgenes.[159] The role of TNF in this model has been confirmed using monoclonal antibodies. This model may be particularly useful for assessing efficacy of reagents that are species-specific for human TNF.

Table 4 Efficacy (therapeutic or prophylactic) of approved rheumatoid arthritis (RA) therapies in rat AIA, rat CIA and mouse CIA

	Rat AIA		Rat CIA		Mouse CIA			
Compound	**Prophylactic**	**Therapeutic**	**Prophylactic**	**Therapeutic**	**Prophylactic**	**Therapeutic**	**Mechanism**	**References**
Methotrexate	+	−		+	+		DMARD	Walz *et al*, Sakuma *et al*, Magari *et al*, Jaffee *et al*, Williams *et al*, Neurath *et al*[54–59]
Ciclosporine A	+		+	−	+	−	DMARD	Williams *et al*, Takagishi *et al*, Phadke *et al*, Magari *et al*, Theisen-Popp *et al*, Kaibara *et al*, Cannon *et al*[37 50 51 56 60–62]
Gold compounds	+/−		+/−		−		DMARD	Probert *et al*, Carlson *et al*, Phadke *et al*, Cannon *et al*[36 38 51 63]
Penicillamine	+		+/−		−		DMARD	Probert *et al*, Carlson *et al*, Phadke *et al*, Lewis *et al*, Nishikaku and Koga[36 38 51 64 65]
Prednisone		+		+		+	Corticosteroid	Walz *et al*, Ward and Cloud, Sloboda *et al*, Paska *et al*[54 66 67 68]
Cyclophosphamide	+	−		+/−		+	Antimetabolite	Probert *et al*, Cannon *et al*, Walz *et al*, Cannon *et al*, Sloboda *et al*[36 49 54 62 67]
Indomethacin		+		+		+	NSAID	Probert *et al*, Walz *et al*, Theisen-Popp *et al*, Cannon *et al*, Nishikaku and Koga, Ward and Cloud, Winter and Nuss, Yamaki *et al*, Griswold *et al*, Inou *et al*[36 54 60 62 65 66 69–72]
Naproxen		+ (d10)	+		−		NSAID	Phadke *et al*, Bendele *et al*, Argentieri *et al*, Takashita *et al*[51 73–75]
Celecoxib		+					COX-2 inhibitor	Penning *et al*[76]
Rofecoxib (withdrawn)	+						COX-2 inhibitor	Chan *et al*[77]
Flobufen		+					Dual COX/LT inhibitor	Bulej *et al*[78]
Anakinra		+ (d8)		+		+	IL1 receptor antagonist	Bendele *et al*, Joosten *et al*[22 79 80]
Etanercept (human sTNFR-Ig)		+/−		+		+	TNF blockade	Wooley *et al*, Bendele *et al*, McComb *et al*, Bendele *et al*, Williams *et al*[42 79 81–83]
Abatacept (CTLA4-Ig)			+			+	Blocks T cell costimulation	Williams *et al*, Webb *et al*[63 85]
Toclizumab (MRA)					+		IL6 receptor antagonist*	Takagi *et al*[86]
Approved in Japan:								
Mizoribine			++	++	+		DNA synthesis inhibitor	Kamada *et al*[87 88]
Actarit		+			+		IL2 agonist	Fujisawa *et al*[89]
FK 506 (tacrolimus)		+		+	++	++		Immunosuppressant
Sakuma *et al*, Magari *et al*, Arita *et al*, Takagishi *et al*[55 56 90 91]								
Iguratimod (Y-614)		+				+	COX-2, TNF inhibitor	Inaba *et al*[92]

+, Demonstrated efficacy; +/−, efficacy in some, but not all, studies; −, no effect. Where there is a ++ sign covering prophylactic and therapeutic efficacy, efficacy was demonstrated but it was not possible to determine whether it was prophylactic or therapeutic efficacy from the information available.
*, Positive in monkey CIA. IL6 deficient mice are resistant to CIA.
AIA, adjuvant-induced arthritis; CIA, collagen-induced arthritis; COX, cyclo-oxygenase; DMARD, disease-modifying antirheumatic drug; IL, interleukin; LT, leukotriene; NSAID, non-steroidal anti-inflammatory drug; TNF, tumour necrosis factor.

The SKG mice have a single mutation on the zeta-chain-associated protein kinase (ZAP)-70 gene that leads to the production of arthritogenic T cells.[160] These mice develop T cell mediated arthritis that has many features resembling human RA, including severe arthritis and synovitis involving the digits, ankles and base of the tail. SKG mice also develop extra-articular lesions, such as pneumonitis and dermatitis. The serum of affected mice has high titres of IgG rheumatoid factor and autoantibodies to type II collagen.[161] Mice deficient in the DNase II gene and IFNiR gene (DNase II–/– IFNiR–/– mice) and

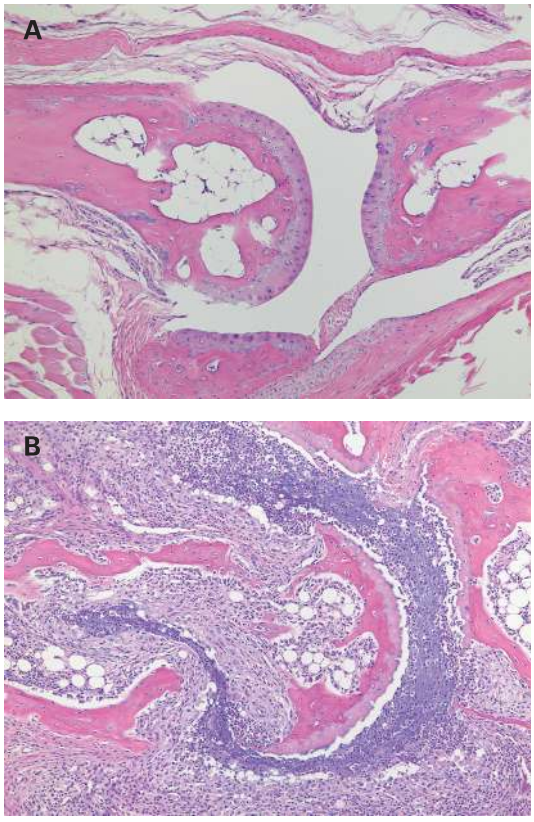

Figure 4 Histology of the ankle joint from a naïve DBA/1 mouse (A) with a normal synovial membrane (one to three synoviocytes thick) and the absence of inflammatory cells is shown in (A). Histology of the ankle joint from a mouse with collagen-induced arthritis (CIA) demonstrating marked inflammatory cell infiltrates, fibrosis and mild to severe cartilage erosion with extension into subchondral bone is shown in (B). Magnification = 100×.

mice with a deletion of the DNase II gene develop a chronic polyarthritis as they age.[162] There is increased expression of the gene encoding TNFα early in the pathogenesis and administration of anti-TNFα antibody has prophylactic and therapeutic efficacy in these mice.

Many of these animal models, particularly some of the mouse models, have been developed recently, thus, the usefulness for predicting efficacy of potential RA therapeutics still needs to be determined.

CONCLUSIONS

Animal models play a critical role in the development of drugs for treating RA. During preclinical evaluation of the efficacy of compounds, it is important to use animal models that are not only appropriate and highly reproducible, but also have been shown to predict clinical efficacy in humans. In this review, we have shown that therapeutic efficacy in animal models seems to be the best predictor of clinical efficacy in human RA. Some of the newer animal models may also prove to be valuable for assessing efficacy of potential therapeutic reagents for RA. Model choice should be performed carefully, taking into account the biology of the animal model and the therapeutic target under evaluation.

It is important to note compounds with activity in the animal models that failed in the clinic may have activity in a subset of patients, such as early RA, but not in the composite RA population. However, due to the population of patients with RA enrolled in the clinical trials, this efficacy could be masked. Moreover, the conclusion that compounds demonstrating therapeutic efficacy in the animal models are more likely to have success in the clinic does not imply that these compounds will be more active if used therapeutically in patients with RA. Indeed, these compounds may be far more effective if used earlier in the disease process. Efforts to understand the early disease processes in RA are invaluable. Identifying or generating animal models that replicate the various mechanisms and pathways of all aspects of RA, including early RA and using these models to test potential therapeutics will help us to make better predictions of efficacy in human RA.

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