ANIMAL MODELS OF RHEUMATOID ARTHRITIS: HOW INFORMATIVE ARE THEY?

Kay McNamee[1], Richard Williams[1] and Michael Seed[2]

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[1]Kennedy Institute of Rheumatology, University of Oxford, Roosevelt Drive, Oxford OX3 7FY, UK

[2]School of Health, Sport and Bioscience, University of East London, Water Lane, London, E15 4LZ, UK

Abstract

Animal models of arthritis are widely used to de-convolute disease pathways and to identify novel drug targets and therapeutic approaches. However, the high attrition rates of drugs in Phase II/III rates means that a relatively small number of drugs reach the market, despite showing efficacy in pre-clinical models. There is also increasing awareness of the ethical issues surrounding the use of animal models of disease and it is timely, therefore, to review the relevance and translatability of animal models of arthritis. In this paper we review the most commonly used animal models in terms of their pathological similarities to human rheumatoid arthritis as well as their response to drug therapy. In general, the ability of animal models to predict efficacy of biologics in man has been good. However, the predictive power of animal models for small molecules has been variable, probably because of differences in the levels of target knockdown achievable in vivo.

1. INTRODUCTION

Animal models of arthritis have been used extensively to test identify drug targets for rheumatoid arthritis (RA) and test potential therapeutics. However, concerns about low clinical development success rates for investigational drugs (Hay et al., 2014), coupled with increasing awareness of the ethical issues surrounding the use of animal models, have led many to question their utility. It is timely, therefore, to review the most commonly used models in terms of their pathological relevance to human RA and in terms of their response to therapeutic intervention.

2. OVERVIEW OF RA

RA affects 0.5-1% of the population in the UK and has a lifetime risk of 4% for women and 2% for men. For many, RA is a painful and disabling disease associated with chronic inflammation. RA is one of the most common causes of disability in the western world with the age of onset typically between 25 and 50, although it can occur at any age. The principal pathological features of the disease include inflammatory erosive synovitis that ultimately leads to destruction of cartilage, bone and soft tissues, resulting in long-term deformity and loss of joint function. Although joints are the main target of the disease process in RA, patients may present with extra-articular features, including sub-cutaneous nodules, vasculitis and pulmonary fibrosis, especially in the more severe cases.

Conventional treatment choices RA include corticosteroids and disease-modifying anti-rheumatic drugs (DMARDs) and for patients who fail to respond adequately to these drugs the additional use of biopharmaceuticals, in particular TNF inhibitors, offers greater opportunities for disease management. However, despite the undoubted success of anti-TNFα, only a quarter of patients treated with a combination of a TNFα inhibitor plus methotrexate achieve disease remission (defined as a DAS-28 score of less than 2.6). In addition, up to 50% of primary responders lose their response within 12 months of the start of therapy (Buch et al., 2007). Hence, there remains a need for more effective anti-arthritic medicines.

The search for new drugs goes hand-in-hand with attempts to understand the aetiopathogenesis of RA and genetic factors are known to play an important role in determining susceptibility to the disease. Indeed, the association between RA susceptibility and specific HLA DRB1 alleles has been demonstrated in a number of populations around the world (Silman and Pearson, 2002). A concept emerged more than a quarter of a century ago suggesting that $CD4^+$ T cells play an important role in the pathogenesis of the disease (Janossy et al., 1981), due to the presence of large numbers of T cells in the joints of RA patients and the association of MHC class II with RA, for which the only known function is to present peptide antigens to $CD4^+$ T cells.

This has developed further with the increased understanding that RA patients present with different synovial pathological features that may relate to different RA phenotypes (Pitzalis et

al., 2013a). The complexities of 'antigen drift' as the disease develops, and accompanying differences in emphasis between cell types, such as CD4+, Th17, and B-cells during this evolution is making RA a disease that has increasing potential for personalised medicine. The prediction of clinical efficacy from RA animal models should therefore be accompanied with a deep understanding of the mechanisms employed and how they relate to the human disease (Pitzalis et al., 2013b).

3. MODELS COMMONLY USED FOR DRUG TESTING

(a) Collagen-induced arthritis (CIA)

Arthritis is induced in susceptible strains of rats and mice by immunisation with type II collagen in incomplete Freund's adjuvant (IFA) or complete Freund's adjuvant (CFA), respectively (Holmdahl et al., 1989; Trentham, 1982). Both T helper (Th) 1 and (Th17) responses are induced in CIA, but Th17 cells appear to play the dominant pathological role (Murphy et al., 2003). The histology of CIA resembles RA in terms of the infiltrating cells in synovial tissue and destruction of bone and cartilage. CIA susceptibility is linked to the I-A region of the *H-2*^{q} and *H-2*^{r} haplotypes in mice. Analysis of the I-A chains of alleles from susceptible and resistant strain (B10.Q) indicated that susceptibility is associated with a four-amino-acid sequence in the I-Aβ chain (Nabozny et al., 1994). This sequence is located in a region associated with binding antigenic peptide, analogous to the genetic susceptibility observed in RA in humans conferred by the DRβ chain. The induction of arthritis in mice of a C57BL/6 (H-2^{b}) background (Campbell et al., 2000; Inglis et al., 2007a) has facilitated the use of gene knockout mice and a more recent development of this has been the generation of the congenic C57Bl/6N.Q strain, that expresses the arthritis susceptible q haplotype of the MHC class II region (Backlund et al., 2013). Rat collagen arthritis is the next most common model used, and is likewise based on the rat equivalent to murine Class 1a, namely MHC Class II RT1 complex, with susceptibility being dominant.

(b) Adjuvant-induced arthritis

The adjuvant-induced arthritis (AA) model was established following the finding that certain strains of rats develop arthritis following administration of CFA (Pearson, 1956). It was initially thought that components of the mycobacteria cross-reacted with joint-specific self-antigens, such as heat shock proteins (van Eden et al., 1988). However, even non-antigenic adjuvants, such as muramyl dipeptide, incomplete Freund's adjuvant and CP20961 can also induce arthritis and it was suggested that these adjuvants may enhance reactivity to self-antigens in the joint (Kohashi et al., 1982). The mechanisms underlying induction of AA are not fully understood but the fact that susceptibility is linked to certain MHC class alleles (Lorentzen and Klareskog, 1996; Vingsbo et al., 1995) and that antibodies to CD4 and MHC class II molecules can inhibit disease (Holmdahl et al., 1992; Larsson et al., 1985) confirms the importance of CD4^{+} T cells. The mechanisms underlying CFA and the mineral oil arthritides are different, with the sensitisation to mycobacterial antigens playing a large role in the former. A difference between adjuvant and collagen arthritis is that resistance is dominant in

adjuvant disease, and MHC playing a lesser role, though still significant. Non-MHC phenotypes contribute significantly, such as the *Aia1, Aia2, and Aia3* regions (Joe et al., 2002). A difference between AA and RA is that AA displays a fairly rapid remission while human RA is a chronic disease. In contrast, arthritis induced by the lipid, pristane (2,6,10,14-tetramethylpentadecane), follows a more chronic relapsing disease course (Bedwell et al., 1987).

(c) Antigen-induced arthritis

Antigen-induced arthritis is seen in mice, rats and rabbits following intra-articular injection of protein antigen (e.g. methylated bovine serum albumin) into the knee joints of animals that have previously immunised with the same antigen (Brackertz et al., 1977; Dumonde and Glynn, 1962).The cellular basis is very similar to CIA, but with more tightly defined sensitisation and challenge steps that can be exploited. It is CD4+ T-cell dependent.The histopathological appearance of antigen-induced arthritis bears similarities to RA, including synovial lining layer hyperplasia, perivascular infiltration with lymphocytes and plasma cells, lymphoid follicles, pannus and cartilage erosions. Indeed repeated injections of antigen can induce ectopic lymphoid structures (ELS) similar in appearance to those seen in RA patient subsets. The erosiveness is related to the ability of the antigen to bind cartilage. However, unlike RA, antigen-induced arthritis is a monoarticular disease that affects only the injected joints. Susceptibility to antigen-induced arthritis is not MHC class II restricted and this makes the model useful for studies involving transgenic and gene knock-out mice.

(d) Bacterial cell wall-induced arthritis

Injection of bacterial cell wall structures can induce arthritis in susceptible strains of rats which is clinically similar to human RA. A single intraperitoneal injection of cell walls may induce a cycle of exacerbation and remission of arthritis. The development of arthritis is thought to be due to accumulation of bacterial cell wall fragments in the joints and once disease is initiated, recurrence can be triggered by microbial superantigens that activate T cells with specific *Vβ* genes in an antigen-independent manner (Schwab et al., 1993).

(e) Spontaneous models

Arthritis occurs spontaneously in mice expressing a modified transgene encoding human TNFα which has been dysregulated by the replacement of the 3' AU rich region with the 3' untranslated region of the human β-globin gene (Keffer et al., 1991). Synovial cells of the joint are a major source of the transgenic TNFα expression.

The K/BxN model arose from transgenic expression of a TCR specific for a peptide from bovine pancreatic ribonuclease. These mice spontaneously developed arthritis when bred on to the NOD background (Kouskoff et al., 1996). Further studies revealed that the development of arthritis in K/BxN mice was dependent on I-A^{g7} MHC class II molecules and could be blocked by treatment non-depleting anti-CD4 mAb (Korganow et al., 1999; Kouskoff et al., 1996; Mangialaio et al., 1999). Although this is highly suggestive that the disease is driven by

$CD4^+$ T cells, it was also found that the development of arthritis required the presence of B lymphocytes (Korganow et al., 1999; Kouskoff et al., 1996). Furthermore, transient arthritis could be transferred by injecting naive mice with serum IgG from arthritic mice in a complement-dependent and FcγR-dependent manner, indicating the pathological role played by autoantibodies in this model (Corr and Crain, 2002; Korganow et al., 1999; Solomon et al., 2002). The molecular target of the autoantibodies was identified as glucose-6-phosphate isomerase (GPI), a ubiquitous cytoplasmic enzyme in the context of $I\text{-}A^{g7}$ MHC class II molecules (Matsumoto et al., 1999).

Sakaguchi et al described a model of arthritis that occurred spontaneously in mice carrying a point mutation of the gene encoding ZAP-70, a key signal transduction molecule in T cells. It was proposed that altered T-cell receptor signalling as a result of aberrant ZAP-70 leads to a failure in thymic deletion and the emergence of autoimmune T cells (Sakaguchi et al., 2003).

4. How do animal models compare pathologically to human RA?

The pathology of RA is not confined to the joint and involves complex pathways and multiple tissues. It is therefore imperative that any animal model chosen for investigation mimics enough of the human condition to be translatable. With a wide variety of models available, a thorough comparison is required to establish which model is appropriate for the research in question. All models discussed here exhibit classical features of RA, namely joint swelling, synovitis, pannus formation, and bone erosion (Table 1). However, each model differs in speed of disease onset, chronicity, severity, resolution and histopathology. Models such as hTNF transgenic mice (Keffer et al., 1991) or K/BxN mice (Kouskoff et al., 1996) develop spontaneous arthritis, potentially providing insights into disease triggers. In contrast, CIA is induced by collagen immunisation and is useful for assessing drug efficacy in a controlled manner. CIA is also useful for investigating the role of specific genes through the use of knockout mice (reviewed (Vincent et al., 2012)). Zap70 mutant SKG mice may develop arthritis spontaneously dependent on environmental factors or may require induction via zymosan administration (Yoshitomi et al., 2005).

The histopathology of the rodent models also differs between each other, as well as with human RA (Patel et al., 2010). Most, including CIA and K/BxN, exhibit a periostitis with accompanying bone shaft involvement, erosion and proliferation. This is especially so in rat adjuvant arthritis (Patel et al., 2010). There is a heavy neutrophil involvement in rodent models, more so than human RA. In CIA the patterns of erosion are similar with erosion progressing from the joint margins through the underlying bone and over the cartilage surface. In rat adjuvant arthritis subchondral bone is degraded by a heavy neutrophilic invasion, depleting cartilage of matrix, but only eroding cartilage in the chronic fibrotic phase. It can be termed a periarthritis. Pristane arthritis has added features of neutrophil aggregates, not seen in either CFA or CIA arthritis. The adjuvant disease results in profound ankylosis in the chronic phases. Antigen induced arthritis progresses in a fashion similar to RA, and repeated injections of antigen induce ectopic lymphoid aggregates as seen in RA subsets.

The disease course in RA is usually progressive and persistent but it is common to have periods of flare and remission. Spontaneous models that reflect this are often more chronic and with a delayed disease onset but with the exception of K/BxN serum transfer (Ditzel, 2004) there is no remission of disease. The hTNF transgenic mouse in particular is extremely destructive and erosive (Keffer et al., 1991; Probert et al., 1996). This is in contrast to CIA, which is acute but remitting in late disease (around 10-14 days post onset) (Mauri et al., 1996).

RA is a systemic disease and as such, multiple tissues can be affected. Most commonly extra-articular manifestations of RA are found in the lungs (interstitial lung disease/fibrosis), heart (arrhythmias and endocarditis) and eyes (ocular inflammation and secondary Sjörgen's syndrome) (reviewed in (Cojocaru et al., 2010; Prete et al., 2011)). This has led to further investigation of the major animal models used in arthritis research to examine whether they also display some or all of this additional pathologies. Following immunisation with type II collagen, DBA/1 mice exhibited signs of pulmonary inflammation characterized by cellular infiltration and cytokine production but it did not correlate with arthritis score. This was largely due to CFA, as immunising with CFA alone without collagen was sufficient to induce lung inflammation. Under the same conditions there was no evidence for any cardiac pathologies or complications (Schurgers et al., 2012). The hTNF model has high systemic inflammation due to over expression of TNF with some evidence that there is lung and cardiac inflammation but this seems to be after targeting these tissues directly rather than an effect of the arthritis model (Franco et al., 1999; Fujita et al., 2001; Tang et al., 2004). Little is known about extra-articular manifestations in the K/BxN model, however there are reports of spontaneous endocarditis alongside arthritis in these mice, interestingly via different pathways (Binstadt et al., 2009). There is also evidence of interstitial lung disease in the zymosan-induced SKG model. Arthritic mice also develop pulmonary fibrosis that is similar to the interstitial lung disease found in some RA patients (Keith et al., 2012). There is limited understanding of whether these additional manifestations are responsive to anti-arthritic treatments and there is scope to further investigate multiple tissue pathology in these models.

Rat CFA arthritis is also a systemic disease, with connective tissue inflammation involving eyes, meninges, skin, bone marrow, increased gut permeability, splenomegaly, and granulomatous liver disease (Patel et al., 2010).

Chronic pain is a prominent feature of RA and pain relief is an unmet need in many patients. Animal models of arthritis have been used to assess pain and analgesics. In CIA, disease onset results in a decrease in threshold to mechanical and thermal stimuli when testing evoked pain response, making it a suitable model to test analgesics. Indeed, treating with anti-TNF from onset in CIA is analgesic as well an anti-arthritic, leading to normalisation of pain and spontaneous behaviour responses (Inglis et al., 2007b). Using the K/BxN serum transfer model to assess joint pain in mice, a reduction in thermal and mechanical threshold was displayed that correlated with joint swelling. Thermal latency returned to control levels

with resolution of disease but mechanical sensitivity persisted beyond the inflammatory phase (Christianson et al., 2012). These models therefore provide a valuable opportunity to investigate persistent sensitisation in arthritis as even with successful therapy, many patients continue to require pain relief. CFA arthritis, or simply CFA injection into the paw, is used in RA pain research, with spinal indices of hyperalgesia being demonstrated, thermal and mechanical thresholds also being reduced.

5. Immune system involvement in RA and animal models: how do they compare?

The aetiology of RA is remains elusive, but it is accepted that genetic susceptibility and environmental triggers are important in causing altered immune function, resulting in autoimmunity.

The presence of autoantibodies is a predominant feature of RA. Rheumatoid factor (RF) is found in 70-90% of RA patients but is also detected in healthy individuals and patients with other autoimmune disorders (Dorner et al., 2004; Dorner and Hansen, 2004). The presence of anti-citrullinated protein antibodies (ACPA) is also highly specific to RA (over 90%) and linked with a more progressive, destructive arthritis (Klareskog et al., 2008; Silveira et al., 2007; Snir et al., 2010). ACPA have been described up to 10 years before disease onset and are additionally correlated with RA risk factors such as smoking and periodontal disease (Hensvold et al., 2015; Mahdi et al., 2009; Wegner et al., 2010). RF is generally thought to be absent from rodent models, but aggressive erosive disease is linked to the presence of anti-citrullinated cyclic peptide antibodies (anti-CCP) which may be generated within RA pannus ELS. CIA mice have been found to develop measurable levels of antibodies specific for certain citrullinated epitopes (Vossenaar et al., 2003). Indeed peptidyl arginine deiminase (PAD) that generates citrullinated peptides, is expressed in CIA synovia, and citrullination of CII enhances the arthritogenic activity (Lundberg et al., 2005). Both ACPA and RF have been described in the CIA model in C57BL/6Q mice (Forster et al., 2012) and in SKG mice (Hsu et al., 2009) (Keith et al., 2013) but there are no reports of ACPA in KxB/N or hTNF mice.

The major histocompatibility complex (MHC) is a gene family separated into 3 classes. Class II molecules are found on APCs and govern presentation of antigens to CD4+ T cells. RA susceptibility is associated with specific alleles within MHC class II, namely *HLA DRB1* (Newton et al., 2004a; Newton et al., 2004b). The frequency of *HLA DRB1* alleles is increased in patients with RA when compared to healthy controls (McInnes and Schett, 2007). These alleles have a common sequence termed the shared epitope, and around 90% of RA patients are positive for this sequence (Gregersen et al., 1987). As in human RA, MHC class II molecules are known to determine disease susceptibility in many animal models of arthritis. The SKG model is MHC class II linked (Sakaguchi et al., 2003), and CIA is normally carried out in mice bred onto specific MHC susceptible haplotype backgrounds (I-Aq is the most common) (Williams, 2007). CIA rats share some susceptibility haplotypes with human arthritic haplotypes (Watson et al., 1990). The MHC phenotype is not as strong in CFA and adjuvant arthritis, in pristane arthritis susceptibility results from negative associations such as Rt1n and

Rt1h. However, heterogenous nuclear ribonucleoprotein A2 (hnRNP-A2) has been identified from epitope screening of RA associated auto-antigens, which indicates a reliance on MHC. In CFA, MHC quantitative trait loci such as *Aia2,* and *Aia3* on chromosome 4 are important. *Aia1, 2 and 3* contain autoimmunity candidate genes, for example *Aia3* : RA2.

KRN mice on an I-Ak background do not develop arthritis, but when crossed with animals expressing I-Ag7 molecules, develop disease (Ditzel, 2004). Although MHC class II genes are important, non-MHC genes are also suggested to be required (Kobezda et al., 2014).

MHC class II molecules present antigens to CD4+ T cells and these cells are present in the synovium of inflamed joints in both humans and animals (Alzabin and Williams, 2011; Todd et al., 1988). The requirement for CD4+ T cells in the initiation of arthritis has been demonstrated in CIA by anti-CD4 mAb treatment prior to onset, however it does not ameliorate established disease (Williams and Whyte, 1996), a result reflected in human clinical trials (Keystone, 2002). This failure to ameliorate established disease is likely to be due to the depletion of all CD4 subsets, including regulatory T cells. As depletion of CD4+ T cells fails to halt joint destruction, the focus has shifted to specific CD4+ T cell populations. Th1 and Th17 cells are defined by their expression of IFN-γ and IL-17, respectively. IFN-γ and IL-17 are found to be elevated in the circulation and inflamed joints of RA patients and many animal models of arthritis and targeting IL-17 has proved therapeutic in CIA (Lubberts et al., 2004) (Nakae et al., 2003), K/BxN mice (Jacobs et al., 2009) and particularly SKG mice in which disease is strongly driven by Th17 cells (Hirota et al., 2007) although the results of IL-17 blockade in human RA have been relatively disappointing (Genovese et al., 2013). Th17 and Th1 cells paradoxically increase in RA patients following anti-TNF therapy, an observation first seen in the CIA model (Notley et al., 2008). Blockade of TNF results in an increase in the IL-12/IL-23 subunit p40 (IL-12 and IL-23 are important for Th1 and Th17 survival, respectively) and further studies have linked increased Th17 and Th1 cells with non-response to anti-TNF in RA patients (Alzabin et al., 2012; Chen et al., 2011).

Regulatory T (CD4+CD25+FOXP3+) cells are known to be defective in RA (Ehrenstein et al., 2004) with a reduced capability to suppress inflammatory cytokine production and increased apoptosis (Toubi et al., 2005). Many models of arthritis have shown that these cells are disease modifying. Thus, depletion of Tregs exacerbates disease and adoptively transferring Tregs into CIA is therapeutic (Morgan et al., 2005). Arthritis in K/BxN mice without functional *foxp3* is more destructive (Nguyen et al., 2007). Anti-TNF restores Treg suppressor function and prevents apoptosis of these cells but there is conflicting evidence with some studies demonstrating an additional protective role for TNF. In support of this, FoxP3 intensity increased following longer TNF exposure in co-cultures of Tregs and T effector cells. This resulted in enhanced suppressive activity and reduction of cytokines via TNFRII (Chen et al., 2007). These conflicting reports and the suggested involvement of TNFRII have led to a focus on the TNF receptors and Tregs in animal models of arthritis. A recent study in CIA highlighted a divergent role for TNF receptors in Tregs. Thus, selective blockade of TNFR1

resulted in expansion of Treg cells and increased suppressive function (including significant reductions in IL-17 and IFN-γ) not seen when targeting both receptors using TNFR-Fc (McCann et al., 2014). It was also suggested that by preserving TNFRII, the resolution of disease is enhanced, associated with increased *foxp3* expression in the joints. Whether a there is a role for TNFRII in human Tregs remains to be established but certainly merits further investigation.

6. Comparison of drug therapy in RA and animal models

(a) Biologics

Anti-TNF has been enormously effective in RA and has transformed the therapeutic landscape. It was the discovery that synovial explants from RA patients spontaneously produced TNF-α and that blockade of TNF in culture demonstrated that production of other pro-inflammatory proteins implicated in RA (IL-1β and GM-CSF) was dependent on TNF, making it a pivotal mediator of disease (Brennan et al., 1989; Haworth et al., 1991). Using the CIA model of arthritis, neutralising TNF was found to ameliorate inflammation and impede joint destruction (Williams et al., 1992). In addition, administration of anti-TNF antibodies to hTNF transgenic mice reduced TNF-induced mortality (Siegel et al., 1995). The clear efficacy of anti-TNF in the models translated into the clinic, with around 70% of patients reporting a 20% or more reduction in symptoms (Maini et al., 1998).

A proportion of patients respond poorly to anti-TNF and focus has now returned to the models to reassess anti-TNF therapy and identify novel biologic targets that could be tested in patients. As discussed previously here, IL-17 was established as a potential target through the CIA and SKG models but lacked efficacy in human RA (Genovese et al., 2013). Ant-IL-17 mAb therapy continues to be explored as a therapy for RA, particularly for anti-TNF non-responders and in other related autoimmune disorders such as psoriasis and anklosing spondylitis. IL-6 is known to promote Th17 differentiation (Bettelli et al., 2006; Korn et al., 2009) and elevated levels of IL-6 have been found in the synovial fluid of RA patients (Hirano et al., 1988; Houssiau et al., 1988; Robak et al., 1998). The joints of SKG mice have enhanced IL-6 gene expression and protein when compared to BALB/c mice and IL-$6^{-/-}$ SKG mice are protected from synovitis and joint destruction and fail to produce Th17 cells (Hirota et al., 2007). Blockade of IL-6 is also efficacious when administered early in CIA (Fujimoto et al., 2008) and anti-IL-6 therapy (tocilizumab) has been successful in multiple trials and is approved for clinic use for RA patients reviewed in (Navarro-Millan et al., 2012).

In addition to blockade of pro-inflammatory cytokines, there are also biologics that target T cell activation, most notably abatacept. Abatacept disrupts the interaction between CD28 (expressed on T cells) and CD80 and CD86 (expressed on APCs) using a enhanced binding homolog of CD28, CTLA-4. Abatacept is made from the extracellular portion of CTLA-4 fused to human IgG1 Fc (CTLA-4Ig) (Bevaart et al., 2010). When given to rats or mice prophylactically, CTLA-4Ig supresses disease onset in CIA and reduces proliferating T cells in the lymph nodes draining the site of immunisation (Knoerzer et al., 1995; Webb et al., 1996).

CTLA-4Ig was also found to be efficacious in ameliorating clinical score when the treatment was given after disease onset (Knoerzer et al., 1995). In RA patients, abatacept is therapeutic in a number of trial conditions including anti-TNF non-responders (Schiff, 2011).

Another validated approach in human RA is anti-B cell (anti-CD20) mAb therapy, which is administered primarily to anti-TNF non-responders (Tak et al., 2012). Although anti-CD20 treatment has not been tested specifically in animal models, B cell deletion or depletion is known to protect against arthritis (Svensson et al., 1998; Yanaba et al., 2007) and anti-CD19 mAb therapy was found to effective in CIA, without depleting B cells (Cemerski et al., 2010).

Animal models of arthritis have undoubtedly facilitated the positive progress of biologics from the lab to the clinic and in general, concordance has been observed between animal models and human disease.

(b) Small molecules

Despite the success of biologics to treat RA, they remain expensive to produce and may only be administered intravenously or subcutaneously (Liu et al., 2012). To address this, small molecular inhibitors (SMIs) have been investigated due to their lower manufacturing costs and oral availability (Stanczyk et al., 2008). SMIs are compounds with a molecular weight less than 1kDa and those targeting inflammation are largely kinase inhibitors. The rationale for kinase inhibition is that they often act upstream of inflammatory mediators including TNF. Mitogen-activated protein kinases (MAPKs) are a well-described pathway involved in inflammation. They comprise three groups; p38, c-Jun N-terminal kinase (JNK) and extracellular-regulated protein kinase (ERK) with p38 the most explored for treatment potential. Inhibition of various p38 inhibitors proved therapeutic in CIA and other models of arthritis, but numerous p38 inhibitors did not progress past phase III RA trials with both toxicity and poor efficacy cited for their failure in the clinic (Hammaker and Firestein, 2010). Tyrosine kinases (JAK and Syk) were the next front runners to emerge from animal models (largely rat and mouse CIA) but with mixed success in the clinic. The JAK family antagonist tofacitinib compared favourably against methotrexate and anti-TNF but due to concerns over the safety profile, it is currently not approved for RA treatment in Europe but is FDA-approved in the US (Cutolo and Meroni, 2013; Garber, 2011). Fostmatinib inhibits Syk kinases important for immune cell signalling as well as osteoclastogensis. After phase III trials in RA, fostmatinib failed to match the efficacy of anti-TNF and is not currently expected to progress further (Fleischmann, 2015).

Animal models as then used have shown to be poor predictors of efficacy for some kinase inhibitors but other SMIs are proving more fruitful, particularly some type 4 phosphodiesterases (PDE4) inhibitors. PDE4 hydrolyses cAMP to AMP and is expressed in immune cells (Page and Spina, 2011). Targeting PDE4 has been shown to reduce TNF in vitro via the protein kinase pathway (PKA) due to the elevation in cAMP (Seldon et al., 1995). Although their anti-inflammatory potential is promising, toxicity issues have stopped some PDE4 inhibitors from reaching the clinic (Palfreeman et al., 2013). Apremilast is a novel PDE4

inhibitor that has been investigated for its tolerability and efficacy in mouse CIA and collagen antibody induced arthritis (CAIA) and significantly reduced clinical score and joint pathology in both. Naïve mice showed no adverse behavioural changes when treated with apremilast in contrast to another PDE4 inhibitor, rolipram, where mice exhibited significant lethargy (McCann et al., 2010). Apremilast has shown efficacy in RA as a monotherapy with only mild adverse events and has been FDA approved for psoriatic arthritis.

Although all animal models of disease will have limitations, their similarities and dissimilarities have furthered our understanding of RA and facilitated transfer of a number of successful therapies to the clinic. Despite this, the high attrition rate of small molecules in Phase III clinical trials (often due to adverse effects not predicted from animal toxicology) gives cause for concern and suggests that pre-clinical studies, particularly with small molecules, should be interpreted with more caution.

Drugs are administered in animal models at doses that take no consideration of long term toxicity. However, dosing and target therapeutic levels in clinical trial necessarily take a more cautious approach. The failure of the early p38 trials appears to relate to the degree of kinase inhibition achieved in the clinic, as opposed to the animal models. The latest generation are focussing on upstream kinases, or dual-site p38 inhibitors designed for enhanced inhibitory activity (Wrobleski et al., 2013). BMS-582949, an example of the latter, does not suffer from the clinical tachypylaxis of the first generation, but did from side effects that could not be modelled in animals (Liu et al., 2013). Dual Inhibition of p38 kinase activation and activity provides efficacy in treatment of rheumatoid arthritis. (Schieven, 2012). The use of biologics (developed using animal models) has raised the benchmark for clinical efficacy and now it is seen that drugs which would have been ground breaking one to two decades ago, are now considered not to reach the efficacy required.

This clinical judgment can now be made following the introduction of clinical assessment tools such as the ACR and EULAR response criteria, enabling the comparison between clinical trials. Animal modelling has not attained this level of sophistication, and in isolation is currently unable to make that level of prediction. Where animal modelling contributes, is when the degree of target knockdown is related to efficacy and drug exposure. Taking into account toxicity, this can be translated to the clinic, calibrating and adapting doses against biomarkers of efficacy against the target.

7. THE 3R's

Animal models of arthritis will continue to be used in drug development and it is important that implementation of the 3R's; replacement, reduction and refinement, is encouraged within research establishments and companies and by regulators, funding bodies and journals. A Joint Working Group held recently in London on the application of the three R's to animal models of arthritis identified the following priority areas.

- Further research into the use of analgesia, with respect to suitable agents, effects on welfare and the science, timing of administration and self-administration.
- Improved indicators of pain and distress, such as accessible computer-assisted behavioural analysis.
- Less severe models, e.g. not requiring the use of potentially severe inducers such as CFA.
- More physiologically relevant spontaneous models using GA mice, which will decrease the number of procedures because it will not be necessary to induce RA.
- International guidelines for refined experimental protocols, including humane endpoints.
- Better sharing and publication of all Three Rs in RA studies.
- Greater support for the development and uptake of *in vitro*, *in silico* and epidemiological approaches to RA research.

8. Conclusions

Animal models of arthritis continue to play an important role in pre-clinical research, particularly for the identification and validation of drug targets. However, their predictive power for the levels of efficacy now required in the clinic is variable. They also appear unable to predict long term adverse effects (e.g. infection risk). which often emerge in large-scale Phase III clinical trials. The use of models requires more considered approaches to integration into the drug development process, especially with respect to calibrating the degree of target knockdown to efficacy and then safety. The application of the Three R's, particularly towards the refinement of existing models of arthritis, remains an important priority for the future.

References

Alzabin, S., Abraham, S.M., Taher, T.E., Palfreeman, A., Hull, D., McNamee, K.E., Jawad, A., Pathan, E., Kinderlerer, A., Taylor, P.C., Williams, R.O., Mageed, R., 2012. Incomplete response of inflammatory arthritis to TNFα blockade is associated with the Th17 pathway. Ann. Rheum. Dis. 71, 1741-1748.

Alzabin, S., Williams, R.O., 2011. Effector T cells in rheumatoid arthritis: lessons from animal models. FEBS Lett. 585, 3649-3659.

Backlund, J., Li, C., Jansson, E., Carlsen, S., Merky, P., Nandakumar, K.S., Haag, S., Ytterberg, J., Zubarev, R.A., Holmdahl, R., 2013. C57BL/6 mice need MHC class II Aq to develop collagen-induced arthritis dependent on autoreactive T cells. Ann. Rheum. Dis. 72, 1225-1232.

Bedwell, A.E., Elson, C.J., Hinton, C.E., 1987. Immunological involvement in the pathogenesis of pristane-induced arthritis. Scand. J. Immunol. 25, 393-398.

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T.B., Oukka, M., Weiner, H.L., Kuchroo, V.K., 2006. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 441, 235-238.

Bevaart, L., Vervoordeldonk, M.J., Tak, P.P., 2010. Evaluation of therapeutic targets in animal models of arthritis: how does it relate to rheumatoid arthritis? Arthritis Rheum. 62, 2192-2205.

Binstadt, B.A., Hebert, J.L., Ortiz-Lopez, A., Bronson, R., Benoist, C., Mathis, D., 2009. The same systemic autoimmune disease provokes arthritis and endocarditis via distinct mechanisms. Proc. Natl. Acad. Sci. U. S. A. 106, 16758-16763.
Brackertz, D., Mitchell, G.F., Mackay, I.R., 1977. Antigen-induced arthritis in mice. I. Induction of arthritis in various strains of mice. Arthritis Rheum. 20, 841-850.
Brennan, F.M., Chantry, D., Jackson, A.M., Maini, R.N., Feldmann, M., 1989. Cytokine production in culture by cells isolated from the synovial membrane. J. Autoimmun. 2 Suppl, 177-186.
Buch, M.H., Bingham, S.J., Bryer, D., Emery, P., 2007. Long-term infliximab treatment in rheumatoid arthritis: subsequent outcome of initial responders. Rheumatology (Oxford) 46, 1153-1156.
Campbell, I.K., Hamilton, J.A., Wicks, I.P., 2000. Collagen-induced arthritis in C57BL/6 (H-2b) mice: new insights into an important disease model of rheumatoid arthritis. Eur. J. Immunol. 30, 1568-1575.
Cemerski, Saso, Chu, C.Q., Seung, Y., Pong, Erik, Horton, Holly, M., Desjarlais, John R., Szymkowski, David, E., 2010. Suppression of collagen-induced arthritis in mice by anti-CD19 antibody that co-engages BCR and inhibitory receptor FcRIIb: efficacy does not require B cell depletion. Arthritis Rheum. 62.
Chen, D.Y., Chen, Y.M., Chen, H.H., Hsieh, C.W., Lin, C.C., Lan, J.L., 2011. Increasing levels of circulating Th17 cells and interleukin-17 in rheumatoid arthritis patients with an inadequate response to anti-TNF-alpha therapy. Arthritis Res Ther 13, R126.
Chen, X., Baumel, M., Mannel, D.N., Howard, O.M., Oppenheim, J.J., 2007. Interaction of TNF with TNF receptor type 2 promotes expansion and function of mouse CD4+CD25+ T regulatory cells. J. Immunol. 179, 154-161.
Christianson, C.A., Corr, M., Yaksh, T.L., Svensson, C.I., 2012. K/BxN serum transfer arthritis as a model of inflammatory joint pain. Methods Mol Biol 851, 249-260.
Cojocaru, M., Cojocaru, I.M., Silosi, I., Vrabie, C.D., Tanasescu, R., 2010. Extra-articular Manifestations in Rheumatoid Arthritis. Maedica 5, 286-291.
Corr, M., Crain, B., 2002. The role of FcgammaR signaling in the K/B x N serum transfer model of arthritis. J. Immunol. 169, 6604-6609.
Cutolo, M., Meroni, M., 2013. Clinical utility of the oral JAK inhibitor tofacitinib in the treatment of rheumatoid arthritis. Journal of inflammation research 6, 129-137.
Ditzel, H.J., 2004. The K/BxN mouse: a model of human inflammatory arthritis. Trends in molecular medicine 10, 40-45.
Dorner, T., Egerer, K., Feist, E., Burmester, G.R., 2004. Rheumatoid factor revisited. Curr. Opin. Rheumatol. 16, 246-253.
Dorner, T., Hansen, A., 2004. Autoantibodies in normals--the value of predicting rheumatoid arthritis. Arthritis Res Ther 6, 282-284.
Dumonde, D.C., Glynn, L.E., 1962. The production of arthritis in rabbits by an immunological reaction to fibrin. British Journal of Experimental Pathology 43, 373-383.
Ehrenstein, M.R., Evans, J.G., Singh, A., Moore, S., Warnes, G., Isenberg, D.A., Mauri, C., 2004. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFalpha therapy. J. Exp. Med. 200, 277-285.
Fleischmann, R.M., 2015. Rheumatoid arthritis: Developing new oral targeted therapies for RA can be challenging. Nat Rev Rheumatol 11, 4-6.
Forster, M., Raposo, B., Ekman, D., Klaczkowska, D., Popovic, M., Nandakumar, K.S., Lindvall, T., Hultqvist, M., Teneva, I., Johannesson, M., Ahlqvist, E., Holmdahl, R., 2012. Genetic control of antibody production during collagen-induced arthritis development in heterogeneous stock mice. Arthritis Rheum. 64, 3594-3603.
Franco, F., Thomas, G.D., Giroir, B., Bryant, D., Bullock, M.C., Chwialkowski, M.C., Victor, R.G., Peshock, R.M., 1999. Magnetic resonance imaging and invasive evaluation of development of heart failure in transgenic mice with myocardial expression of tumor necrosis factor-alpha. Circulation 99, 448-454.
Fujimoto, M., Serada, S., Mihara, M., Uchiyama, Y., Yoshida, H., Koike, N., Ohsugi, Y., Nishikawa, T., Ripley, B., Kimura, A., Kishimoto, T., Naka, T., 2008. Interleukin-6 blockade suppresses autoimmune arthritis in mice by the inhibition of inflammatory Th17 responses. Arthritis Rheum. 58, 3710-3719.
Fujita, M., Shannon, J.M., Irvin, C.G., Fagan, K.A., Cool, C., Augustin, A., Mason, R.J., 2001. Overexpression of tumor necrosis factor-alpha produces an increase in lung volumes and

pulmonary hypertension. American journal of physiology. Lung cellular and molecular physiology 280, L39-49.
Garber, K., 2011. Pfizer's JAK inhibitor sails through phase 3 in rheumatoid arthritis. Nature biotechnology 29, 467-468.
Genovese, M.C., Durez, P., Richards, H.B., Supronik, J., Dokoupilova, E., Mazurov, V., Aelion, J.A., Lee, S.H., Codding, C.E., Kellner, H., Ikawa, T., Hugot, S., Mpofu, S., 2013. Efficacy and safety of secukinumab in patients with rheumatoid arthritis: a phase II, dose-finding, double-blind, randomised, placebo controlled study. Ann. Rheum. Dis. 72, 863-869.
Gregersen, P.K., Silver, J., Winchester, R.J., 1987. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. Arthritis Rheum. 30, 1205-1213.
Hammaker, D., Firestein, G.S., 2010. "Go upstream, young man": lessons learned from the p38 saga. Ann. Rheum. Dis. 69 Suppl 1, i77-82.
Haworth, C., Brennan, F.M., Chantry, D., Turner, M., Maini, R.N., Feldmann, M., 1991. Expression of granulocyte-macrophage colony-stimulating factor in rheumatoid arthritis: regulation by tumor necrosis factor-a. Eur. J. Immunol. 21, 2575-2579.
Hay, M., Thomas, D.W., Craighead, J.L., Economides, C., Rosenthal, J., 2014. Clinical development success rates for investigational drugs. Nature biotechnology 32, 40-51.
Hensvold, A.H., Magnusson, P.K., Joshua, V., Hansson, M., Israelsson, L., Ferreira, R., Jakobsson, P.J., Holmdahl, R., Hammarstrom, L., Malmstrom, V., Askling, J., Klareskog, L., Catrina, A.I., 2015. Environmental and genetic factors in the development of anticitrullinated protein antibodies (ACPAs) and ACPA-positive rheumatoid arthritis: an epidemiological investigation in twins. Ann. Rheum. Dis. 74, 375-380.
Hirano, T., Matsuda, T., Turner, M., Miyasaka, N., Buchan, G., Tang, B., Sato, K., Shimizu, M., Maini, R., Feldmann, M., et al., 1988. Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis. Eur. J. Immunol. 18, 1797-1801.
Hirota, K., Hashimoto, M., Yoshitomi, H., Tanaka, S., Nomura, T., Yamaguchi, T., Iwakura, Y., Sakaguchi, N., Sakaguchi, S., 2007. T cell self-reactivity forms a cytokine milieu for spontaneous development of IL-17+ Th cells that cause autoimmune arthritis. J. Exp. Med. 204, 41-47.
Holmdahl, R., Andersson, M.E., Goldschmidt, T.J., Jansson, L., Karlsson, M., Malmström, V., Mo, J., 1989. Collagen induced arthritis as an experimental model for rheumatoid arthritis. Immunogenetics, pathogenesis and autoimmunity. APMIS 97, 575-584.
Holmdahl, R., Goldschmidt, T.J., Kleinau, S., Kvick, C., Jonsson, R., 1992. Arthritis induced in rats with adjuvant oil is a genetically restricted, alpha beta T-cell dependent autoimmune disease. Immunology 76, 197-202.
Houssiau, F.A., Devogelaer, J.P., Van Damme, J., de Deuxchaisnes, C.N., Van Snick, J., 1988. Interleukin-6 in synovial fluid and serum of patients with rheumatoid arthritis and other inflammatory arthritides. Arthritis Rheum. 31, 784-788.
Hsu, L.Y., Tan, Y.X., Xiao, Z., Malissen, M., Weiss, A., 2009. A hypomorphic allele of ZAP-70 reveals a distinct thymic threshold for autoimmune disease versus autoimmune reactivity. J. Exp. Med. 206, 2527-2541.
Inglis, J.J., Criado, G., Medghalchi, M., Andrews, M., Sandison, A., Feldmann, M., Williams, R.O., 2007a. Collagen-Induced Arthritis in C57BL/6 mice is associated with a robust and sustained T-cell response to type II collagen. Arthritis Res Ther 9, R113.
Inglis, J.J., Notley, C.A., Essex, D., Wilson, A.W., Feldmann, M., Anand, P., Williams, R., 2007b. Collagen-induced arthritis as a model of hyperalgesia: functional and cellular analysis of the analgesic actions of tumor necrosis factor blockade. Arthritis Rheum. 56, 4015-4023.
Jacobs, J.P., Wu, H.J., Benoist, C., Mathis, D., 2009. IL-17-producing T cells can augment autoantibody-induced arthritis. Proc. Natl. Acad. Sci. U. S. A. 106, 21789-21794.
Janossy, G., Panayi, G., Duke, O., Bofill, M., Poulter, L.W., Goldstein, G., 1981. Rheumatoid arthritis: a disease of T-lymphocyte/macrophage immunoregulation. Lancet 2, 839-842.
Joe, B., Cannon, G.W., Griffiths, M.M., Dobbins, D.E., Gulko, P.S., Wilder, R.L., Remmers, E.F., 2002. Evaluation of quantitative trait loci regulating severity of mycobacterial adjuvant-induced arthritis in monocongenic and polycongenic rats: identification of a new regulatory locus on rat chromosome 10 and evidence of overlap with rheumatoid arthritis susceptibility loci. Arthritis Rheum. 46, 1075-1085.
Keffer, J., Probert, L., Cazlaris, H., Georgopoulos, S., Kaslaris, E., Kioussis, D., Kollias, G., 1991. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. EMBO J. 10, 4025-4031.

Keith, R.C., Powers, J.L., Redente, E.F., Sergew, A., Martin, R.J., Gizinski, A., Holers, V.M., Sakaguchi, S., Riches, D.W., 2012. A novel model of rheumatoid arthritis-associated interstitial lung disease in SKG mice. Experimental lung research 38, 55-66.
Keith, R.C., Sokolove, J., Edelman, B.L., Lahey, L., Redente, E.F., Holers, V.M., Sakaguchi, S., Robinson, W.H., Riches, D.W., 2013. Testosterone is protective in the sexually dimorphic development of arthritis and lung disease in SKG mice. Arthritis Rheum. 65, 1487-1493.
Keystone, E., 2002. Treatments no longer in development for rheumatoid arthritis. Ann. Rheum. Dis. 61 Suppl 2, ii43-45.
Klareskog, L., Ronnelid, J., Lundberg, K., Padyukov, L., Alfredsson, L., 2008. Immunity to citrullinated proteins in rheumatoid arthritis. Annu. Rev. Immunol. 26, 651-675.
Knoerzer, D.B., Karr, R.W., Schwartz, B.D., Mengle-Gaw, L.J., 1995. Collagen-induced arthritis in the BB rat. Prevention of disease by treatment with CTLA-4-Ig. J. Clin. Invest. 96, 987-993.
Kobezda, T., Ghassemi-Nejad, S., Mikecz, K., Glant, T.T., Szekanecz, Z., 2014. Of mice and men: how animal models advance our understanding of T-cell function in RA. Nat Rev Rheumatol 10, 160-170.
Kohashi, O., Aihara, K., Ozawa, A., Kotani, S., Azuma, I., 1982. New model of a synthetic adjuvant, N-acetylmuramyl-L-alanyl-D-isoglutamine- induced arthritis: clinical and histologic studies in athymic nude and euthymic rats. Lab. Invest. 47, 27-36.
Korganow, A.S., Ji, H., Mangialaio, S., Duchatelle, V., Pelanda, R., Martin, T., Degott, C., Kikutani, H., Rajewsky, K., Pasquali, J.L., Benoist, C., Mathis, D., 1999. From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. Immunity 10, 451-461.
Korn, T., Bettelli, E., Oukka, M., Kuchroo, V.K., 2009. IL-17 and Th17 Cells. Annu. Rev. Immunol. 27, 485-517.
Kouskoff, V., Korganow, A.S., Duchatelle, V., Degott, C., Benoist, C., Mathis, D., 1996. Organ-specific disease provoked by systemic autoimmunity. Cell 87, 811-822.
Larsson, P., Holmdahl, R., Dencker, L., Klareskog, L., 1985. In vivo treatment with W3/13 (anti-pan T) but not with OX8 (anti-suppressor/cytotoxic T) monoclonal antibodies impedes the development of adjuvant arthritis in rats. Immunology 56, 383-391.
Liu, C., Lin, J., Everlof, G., Gesenberg, C., Zhang, H., Marathe, P.H., Malley, M., Galella, M.A., McKinnon, M., Dodd, J.H., Barrish, J.C., Schieven, G.L., Leftheris, K., 2013. Synthesis and evaluation of carbamoylmethylene linked prodrugs of BMS-582949, a clinical p38alpha inhibitor. Bioorg Med Chem Lett 23, 3028-3033.
Liu, Y., Wu, E.Q., Bensimon, A.G., Fan, C.P., Bao, Y., Ganguli, A., Yang, M., Cifaldi, M., Mulani, P., 2012. Cost per responder associated with biologic therapies for Crohn's disease, psoriasis, and rheumatoid arthritis. Advances in therapy 29, 620-634.
Lorentzen, J.C., Klareskog, L., 1996. Susceptibility of DA rats to arthritis induced with adjuvant oil or rat collagen is determined by genes both within and outside the major histocompatibility complex. Scand. J. Immunol. 44, 592-598.
Lubberts, E., Koenders, M.I., Oppers-Walgreen, B., van den Bersselaar, L., Coenen-de Roo, C.J., Joosten, L.A., van den Berg, W.B., 2004. Treatment with a neutralizing anti-murine interleukin-17 antibody after the onset of collagen-induced arthritis reduces joint inflammation, cartilage destruction, and bone erosion. Arthritis Rheum. 50, 650-659.
Lundberg, K., Nijenhuis, S., Vossenaar, E.R., Palmblad, K., van Venrooij, W.J., Klareskog, L., Zendman, A.J., Harris, H.E., 2005. Citrullinated proteins have increased immunogenicity and arthritogenicity and their presence in arthritic joints correlates with disease severity. Arthritis Res Ther 7, R458-467.
Mahdi, H., Fisher, B.A., Kallberg, H., Plant, D., Malmstrom, V., Ronnelid, J., Charles, P., Ding, B., Alfredsson, L., Padyukov, L., Symmons, D.P., Venables, P.J., Klareskog, L., Lundberg, K., 2009. Specific interaction between genotype, smoking and autoimmunity to citrullinated alpha-enolase in the etiology of rheumatoid arthritis. Nat Genet 41, 1319-1324.
Maini, R.N., Breedveld, F.C., Kalden, J.R., Smolen, J.S., Davis, D., Macfarlane, J.D., Antoni, C., Leeb, B., Elliott, M.J., Woody, J.N., Schaible, T.F., Feldmann, M., 1998. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum. 41, 1552-1563.
Mangialaio, S., Ji, H., Korganow, A.S., Kouskoff, V., Benoist, C., Mathis, D., 1999. The arthritogenic T cell receptor and its ligand in a model of spontaneous arthritis. Arthritis Rheum. 42, 2517-2523.

Matsumoto, I., Staub, A., Benoist, C., Mathis, D., 1999. Arthritis provoked by linked T and B cell recognition of a glycolytic enzyme. Science 286, 1732-1735.
Mauri, C., Williams, R.O., Walmsley, M., Feldmann, M., 1996. Relationship between Th1/Th2 cytokine patterns and the arthritogenic response in collagen-induced arthritis. Eur. J. Immunol. 26, 1511-1518.
McCann, F.E., Palfreeman, A.C., Andrews, M., Perocheau, D.P., Inglis, J.J., Schafer, P., Feldmann, M., Williams, R.O., Brennan, F.M., 2010. Apremilast, a novel PDE4 inhibitor, inhibits spontaneous production of tumour necrosis factor-alpha from human rheumatoid synovial cells and ameliorates experimental arthritis. Arthritis Res Ther 12, R107.
McCann, F.E., Perocheau, D.P., Ruspi, G., Blazek, K., Davies, M.L., Feldmann, M., Dean, J.L., Stoop, A.A., Williams, R.O., 2014. Selective tumor necrosis factor receptor I blockade is antiinflammatory and reveals immunoregulatory role of tumor necrosis factor receptor II in collagen-induced arthritis. Arthritis Rheumatol 66, 2728-2738.
McInnes, I.B., Schett, G., 2007. Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol 7, 429-442.
Morgan, M.E., Flierman, R., van Duivenvoorde, L.M., Witteveen, H.J., van Ewijk, W., van Laar, J.M., de Vries, R.R., Toes, R.E., 2005. Effective treatment of collagen-induced arthritis by adoptive transfer of CD25+ regulatory T cells. Arthritis Rheum. 52, 2212-2221.
Murphy, C.A., Langrish, C.L., Chen, Y., Blumenschein, W., McClanahan, T., Kastelein, R.A., Sedgwick, J.D., Cua, D.J., 2003. Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. J. Exp. Med. 198, 1951-1957.
Nabozny, G.H., Bull, M.J., Hanson, J., Griffiths, M.M., Luthra, H.S., David, C.S., 1994. Collagen-induced arthritis in T cell receptor V_b congenic B10.Q mice. J. Exp. Med. 180, 517-524.
Nakae, S., Nambu, A., Sudo, K., Iwakura, Y., 2003. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. J. Immunol. 171, 6173-6177.
Navarro-Millan, I., Singh, J.A., Curtis, J.R., 2012. Systematic review of tocilizumab for rheumatoid arthritis: a new biologic agent targeting the interleukin-6 receptor. Clinical therapeutics 34, 788-802 e783.
Newton, J.L., Harney, S.M., Timms, A.E., Sims, A.M., Rockett, K., Darke, C., Wordsworth, B.P., Kwiatkowski, D., Brown, M.A., 2004a. Dissection of class III major histocompatibility complex haplotypes associated with rheumatoid arthritis. Arthritis Rheum. 50, 2122-2129.
Newton, J.L., Harney, S.M., Wordsworth, B.P., Brown, M.A., 2004b. A review of the MHC genetics of rheumatoid arthritis. Genes Immun 5, 151-157.
Nguyen, L.T., Jacobs, J., Mathis, D., Benoist, C., 2007. Where FoxP3-dependent regulatory T cells impinge on the development of inflammatory arthritis. Arthritis Rheum. 56, 509-520.
Notley, C.A., Inglis, J.J., Alzabin, S., McCann, F.E., McNamee, K.E., Williams, R.O., 2008. Blockade of tumor necrosis factor in collagen-induced arthritis reveals a novel immunoregulatory pathway for Th1 and Th17 cells. J. Exp. Med. 205, 2491-2497.
Page, C.P., Spina, D., 2011. Phosphodiesterase inhibitors in the treatment of inflammatory diseases. Handbook of experimental pharmacology, 391-414.
Palfreeman, A.C., McNamee, K.E., McCann, F.E., 2013. New developments in the management of psoriasis and psoriatic arthritis: a focus on apremilast. Drug design, development and therapy 7, 201-210.
Patel, H.B., Dawson, B., Humby, F., Blades, M., Pitzalis, C., Burnet, M., Seed, M., 2010. Animal Models of Rheumatoid Arthritis, in: Serhan, C.N., Ward, P.A., Gilroy, D.W. (Eds.), Fundamentals of Inflammation, Cambridge.
Pearson, C.M., 1956. Development of arthritis, periarthritis and periostitis in rats given adjuvants. Proc. Soc. Exp. Biol. Med. 91, 95-101.
Pitzalis, C., Humby, F., Seed, M.P., 2013a. Synovial Pathology, in: Watts, R.O., Conaghan, P.G., Denton, C., Foster, H., Isaacs, J., Müller-Ladner, U. (Eds.), Oxford Textbook of Rheumatology, 4 ed, Oxford.
Pitzalis, C., Kelly, S., Humby, F., 2013b. New learnings on the pathophysiology of RA from synovial biopsies. Curr. Opin. Rheumatol. 25, 334-344.
Prete, M., Racanelli, V., Digiglio, L., Vacca, A., Dammacco, F., Perosa, F., 2011. Extra-articular manifestations of rheumatoid arthritis: An update. Autoimmun Rev 11, 123-131.
Probert, L., Akassoglou, K., Alexopoulou, L., Douni, E., Haralambous, S., Hill, S., Kassiotis, G., Kontoyiannis, D., Pasparakis, M., Plows, D., Kollias, G., 1996. Dissection of the pathologies induced by transmembrane and wild-type tumor necrosis factor in transgenic mice. J. Leukoc. Biol. 59, 518-525.

Robak, T., Gladalska, A., Stepien, H., Robak, E., 1998. Serum levels of interleukin-6 type cytokines and soluble interleukin-6 receptor in patients with rheumatoid arthritis. Mediators Inflamm 7, 347-353.
Sakaguchi, N., Takahashi, T., Hata, H., Nomura, T., Tagami, T., Yamazaki, S., Sakihama, T., Matsutani, T., Negishi, I., Nakatsuru, S., Sakaguchi, S., 2003. Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. Nature 426, 454-460.
Schieven, G.L., 2012. Dual inhibition of p38 kinase activation and activity provides efficacy in treatment of rheumatoid arthritis. Inflamm. Res. 61, S7.
Schiff, M., 2011. Abatacept treatment for rheumatoid arthritis. Rheumatology (Oxford) 50, 437-449.
Schurgers, E., Mertens, F., Vanoirbeek, J.A., Put, S., Mitera, T., De Langhe, E., Billiau, A., Hoet, P.H., Nemery, B., Verbeken, E., Matthys, P., 2012. Pulmonary inflammation in mice with collagen-induced arthritis is conditioned by complete Freund's adjuvant and regulated by endogenous IFN-gamma. Eur. J. Immunol. 42, 3223-3234.
Schwab, J.H., Brown, R.R., Anderle, S.K., Schlievert, P.M., 1993. Superantigen can reactivate bacterial cell wall-induced arthritis. J. Immunol. 150, 4151-4159.
Seldon, P.M., Barnes, P.J., Meja, K., Giembycz, M.A., 1995. Suppression of lipopolysaccharide-induced tumor necrosis factor-alpha generation from human peripheral blood monocytes by inhibitors of phosphodiesterase 4: interaction with stimulants of adenylyl cyclase. Mol. Pharmacol. 48, 747-757.
Siegel, M.D., Zhang, D.H., Ray, P., Ray, A., 1995. Activation of the interleukin-5 promoter by cAMP in murine EL-4 cells requires the GATA-3 and CLE0 elements. J. Biol. Chem. 270, 24548-24555.
Silman, A.J., Pearson, J.E., 2002. Epidemiology and genetics of rheumatoid arthritis. Arthritis Res 4 Suppl 3, S265-272.
Silveira, I.G., Burlingame, R.W., von Muhlen, C.A., Bender, A.L., Staub, H.L., 2007. Anti-CCP antibodies have more diagnostic impact than rheumatoid factor (RF) in a population tested for RF. Clin. Rheumatol. 26, 1883-1889.
Snir, O., Widhe, M., Hermansson, M., von Spee, C., Lindberg, J., Hensen, S., Lundberg, K., Engstrom, A., Venables, P.J., Toes, R.E., Holmdahl, R., Klareskog, L., Malmstrom, V., 2010. Antibodies to several citrullinated antigens are enriched in the joints of rheumatoid arthritis patients. Arthritis Rheum. 62, 44-52.
Solomon, S., Kolb, C., Mohanty, S., Jeisy-Walder, E., Preyer, R., Schollhorn, V., Illges, H., 2002. Transmission of antibody-induced arthritis is independent of complement component 4 (C4) and the complement receptors 1 and 2 (CD21/35). Eur. J. Immunol. 32, 644-651.
Stanczyk, J., Ospelt, C., Gay, S., 2008. Is there a future for small molecule drugs in the treatment of rheumatic diseases? Curr. Opin. Rheumatol. 20, 257-262.
Svensson, L., Jirholt, J., Holmdahl, R., Jansson, L., 1998. B cell-deficient mice do not develop type II collagen-induced arthritis (CIA). Clin. Exp. Immunol. 111, 521-526.
Tak, P.P., Rigby, W., Rubbert-Roth, A., Peterfy, C., van Vollenhoven, R.F., Stohl, W., Healy, E., Hessey, E., Reynard, M., Shaw, T., 2012. Sustained inhibition of progressive joint damage with rituximab plus methotrexate in early active rheumatoid arthritis: 2-year results from the randomised controlled trial IMAGE. Ann. Rheum. Dis. 71, 351-357.
Tang, Z., McGowan, B.S., Huber, S.A., McTiernan, C.F., Addya, S., Surrey, S., Kubota, T., Fortina, P., Higuchi, Y., Diamond, M.A., Wyre, D.S., Feldman, A.M., 2004. Gene expression profiling during the transition to failure in TNF-alpha over-expressing mice demonstrates the development of autoimmune myocarditis. Journal of molecular and cellular cardiology 36, 515-530.
Todd, J.A., Acha-Orbea, H., Bell, J.I., Chao, N., Fronek, Z., Jacob, C.O., McDermott, M., Sinha, A.A., Timmerman, L., Steinman, L., et al., 1988. A molecular basis for MHC class II--associated autoimmunity. Science 240, 1003-1009.
Toubi, E., Kessel, A., Mahmudov, Z., Hallas, K., Rozenbaum, M., Rosner, I., 2005. Increased spontaneous apoptosis of CD4+CD25+ T cells in patients with active rheumatoid arthritis is reduced by infliximab. Ann. N. Y. Acad. Sci. 1051, 506-514.
Trentham, D.E., 1982. Collagen arthritis as a relevant model for rheumatoid arthritis: evidence pro and con. Arthritis Rheum. 25, 911-916.
van Eden, W., Thole, J.E., Van der Zee, R., Noordzij, A., van Embden, J.D., Cohen, I.R., 1988. Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. Nature 331, 171-173.

Vincent, T.L., Williams, R.O., Maciewicz, R., Silman, A., Garside, P., Arthritis Research, U.K.a.m.w.g., 2012. Mapping pathogenesis of arthritis through small animal models. Rheumatology (Oxford) 51, 1931-1941.
Vingsbo, C., Jonsson, R., Holmdahl, R., 1995. Avridine-induced arthritis in rats; a T cell-dependent chronic disease influenced both by MHC genes and by non-MHC genes. Clin. Exp. Immunol. 99, 359-363.
Vossenaar, E.R., Nijenhuis, S., Helsen, M.M., van der Heijden, A., Senshu, T., van den Berg, W.B., van Venrooij, W.J., Joosten, L.A., 2003. Citrullination of synovial proteins in murine models of rheumatoid arthritis. Arthritis Rheum. 48, 2489-2500.
Watson, W.C., Thompson, J.P., Terato, K., Cremer, M.A., Kang, A.H., 1990. Human HLA-DR beta gene hypervariable region homology in the biobreeding BB rat: selection of the diabetic-resistant subline as a rheumatoid arthritis research tool to characterize the immunopathologic response to human type II collagen. J. Exp. Med. 172, 1331-1339.
Webb, L.M., Walmsley, M.J., Feldmann, M., 1996. Prevention and amelioration of collagen-induced arthritis by blockade of the CD28 co-stimulatory pathway: requirement for both B7-1 and B7-2. Eur. J. Immunol. 26, 2320-2328.
Wegner, N., Wait, R., Sroka, A., Eick, S., Nguyen, K.A., Lundberg, K., Kinloch, A., Culshaw, S., Potempa, J., Venables, P.J., 2010. Peptidylarginine deiminase from Porphyromonas gingivalis citrullinates human fibrinogen and alpha-enolase: implications for autoimmunity in rheumatoid arthritis. Arthritis Rheum. 62, 2662-2672.
Williams, R.O., 2007. Collagen-induced arthritis in mice. Methods Mol Med 136, 191-199.
Williams, R.O., Feldmann, M., Maini, R.N., 1992. Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. Proc. Natl. Acad. Sci. U. S. A. 89, 9784-9788.
Williams, R.O., Whyte, A., 1996. Anti-CD4 monoclonal antibodies suppress murine collagen-induced arthritis only at the time of primary immunisation. Cell. Immunol. 170, 291-295.
Wrobleski, S.T., Lin, S., Dhar, T.G., Dyckman, A.J., Li, T., Pitt, S., Zhang, R., Fan, Y., Doweyko, A.M., Tokarski, J.S., Kish, K.F., Kiefer, S.E., Sack, J.S., Newitt, J.A., Witmer, M.R., McKinnon, M., Barrish, J.C., Dodd, J.H., Schieven, G.L., Leftheris, K., 2013. The identification of novel p38alpha isoform selective kinase inhibitors having an unprecedented p38alpha binding mode. Bioorg Med Chem Lett 23, 4120-4126.
Yanaba, K., Hamaguchi, Y., Venturi, G.M., Steeber, D.A., St Clair, E.W., Tedder, T.F., 2007. B cell depletion delays collagen-induced arthritis in mice: arthritis induction requires synergy between humoral and cell-mediated immunity. J. Immunol. 179, 1369-1380.
Yoshitomi, H., Sakaguchi, N., Kobayashi, K., Brown, G.D., Tagami, T., Sakihama, T., Hirota, K., Tanaka, S., Nomura, T., Miki, I., Gordon, S., Akira, S., Nakamura, T., Sakaguchi, S., 2005. A role for fungal β-glucans and their receptor Dectin-1 in the induction of autoimmune arthritis in genetically susceptible mice. J. Exp. Med. 201, 949-960.

Feature in human RA	CIA	hTNF transgenic	K/BxN	Spontaneous/zymosan induced arthritis in SKG
Joint pathology				
Swelling of multiple joints	**Yes** polyarthritis occurs 21-28 days post immunisation	**Yes** from 4 weeks old	Yes from 4 weeks old in transgenic strain or 7-14 days after serum transfer into naïve mice	Yes by 2 weeks post zymosan injection or 2 months in spontaneous model
Synovitis	**Yes** synovitis is evident by 10 days post disease onset	**Yes** from 4 weeks old	Yes	Yes
Pannus formation	**Yes** pannus is observed by 7-10 days post disease onset	**Yes** by 10 weeks old	Yes	Yes
Cartilage degradation	**Yes** observed by 7-10 days post disease onset	**Yes** by 10 weeks old	Yes	Yes
Bone erosion	**Yes** observed by 10 days post disease onset	**Yes** by 10 weeks old	Yes	Yes
Chronic pain	**Yes** from disease onset, reduced with effective anti-arthritic therapy	Not investigated in this model but likely due to the established role of TNF in promoting inflammatory pain	**Yes** in serum transfer model from disease onset and persists post inflammation resolution	Not investigated but likely due to persistent swelling and redness in the joints
Extra-articular pathololology				
Pulmonary Fibrosis	**Partial** lung involvement (variable and due to CFA) not correlated with clinical score	Some lung involvement but overexpression of TNF is not enough to produce	None reported	Lung disease resembling fibrosis 12 weeks post zymosan i.p injection

		fibrosis without additional insult		
Cardiac involvement	None reported or negative	Yes cardiomyopathy and arrhythmias in cardiac-specific TNF overexpression	Yes cardiac valve inflammation	None reported
Ocular inflammation	None reported	None reported	None reported	None reported
Auto-antibodies				
Citrillinated proteins (ACPA)	Yes (reported in C57BL/6Q mice)	No	No	Yes due to altered thymic selection
Rheumatoid Factor	Yes (reported in C57BL/6Q mice)	No	No	Yes due to altered thymic selection