Animal models for arthritis: innovative tools for prevention and treatment

Kollias, G; Papadaki, P; Apparailly, F; Vervoordeldonk, M J; Holmdahl, R; Baumans, V; Desaintes, C; Di Santo, J; Distler, J; Garside, P; Hegen, M; Huizinga, T W J; Jüngel, A; Klæreskog, L; McInnes, I; Ragoussis, I; Schett, G; Hart, B; Tak, P P; Toes, R; van den Berg, W B; Wurst, W; Gay, S

Postprint available at:
http://www.zora.uzh.ch

Posted at the Zurich Open Repository and Archive, University of Zurich.
http://www.zora.uzh.ch

Originally published at:
Kollias, G; Papadaki, P; Apparailly, F; Vervoordeldonk, M J; Holmdahl, R; Baumans, V; Desaintes, C; Di Santo, J; Distler, J; Garside, P; Hegen, M; Huizinga, T W J; Jüngel, A; Klæreskog, L; McInnes, I; Ragoussis, I; Schett, G; Hart, B; Tak, P P; Toes, R; van den Berg, W B; Wurst, W; Gay, S (2011). Animal models for arthritis: innovative tools for prevention and treatment. Annals of the Rheumatic Diseases:Epub ahead of print.
Animal models for arthritis: innovative tools for prevention and treatment

Abstract

The development of novel treatments for rheumatoid arthritis (RA) requires the interplay between clinical observations and studies in animal models. Given the complex molecular pathogenesis and highly heterogeneous clinical picture of RA, there is an urgent need to dissect its multifactorial nature and to propose new strategies for preventive, early and curative treatments. Research on animal models has generated new knowledge on RA pathophysiology and aetiology and has provided highly successful paradigms for innovative drug development. Recent focus has shifted towards the discovery of novel biomarkers, with emphasis on presymptomatic and emerging stages of human RA, and towards addressing the pathophysiological mechanisms and subsequent efficacy of interventions that underlie different disease variants. Shifts in the current paradigms underlying RA pathogenesis have also led to increased demand for new (including humanised) animal models. There is therefore an urgent need to integrate the knowledge on human and animal models with the ultimate goal of creating a comprehensive 'pathogenesis map' that will guide alignment of existing and new animal models to the subset of disease they mimic. This requires full and standardised characterisation of all models at the genotypic, phenotypic and biomarker level, exploiting recent technological developments in ‘-omics’ profiling and computational biology as well as state of the art bioimaging. Efficient integration and dissemination of information and resources as well as outreach to the public will be necessary to manage the plethora of data accumulated and to increase community awareness and support for innovative animal model research in rheumatology.
Animal models for Arthritis:
Innovative tools for prevention and therapy

George Kollias¹*, Piyi Papadaki¹, Florence Apparailly², Margriet J. Vervoordeldonk³, Rikard Holmdahl⁴, Vera Baumans⁵, Christian Desaintes⁶, James Di Santo⁷, Jörg Distler⁸, Paul Garside⁹, Martin Hegen¹⁰, Tom W. J. Huizinga¹¹, Astrid Jüngel¹², Lars Klareskog³, Iain McInnes⁹, Ioannis Ragoussis¹³, Georg Schett⁸, Bert ’t Hart¹⁴, Paul P. Tak³, Rene Toes¹¹, Wim van den Berg¹⁵, Wolfgang Wurst¹⁶, and Steffen Gay¹²

¹Biomedical Sciences Research Center "Alexander Fleming", Vari-Athens, Greece; ²INSERM, Montpellier, France; ³Academic Medical Center, University of Amsterdam, The Netherlands; ⁴Karolinska Institute, Stockholm, Sweden; ⁵University of Utrecht, Utrecht, The Netherlands; ⁶European Commission, Brussels, Belgium; ⁷Pasteur Institute, Paris, France; ⁸University of Erlangen-Nuremberg, Erlangen, Germany; ⁹University of Glasgow, Glasgow, UK; ¹⁰Pfizer, Cambridge, MA, USA; ¹¹Leiden University Medical Center, Leiden, The Netherlands; ¹²UZH, Zurich, Switzerland; ¹³WTCHG, University of Oxford, Oxford, UK; ¹⁴BPRC, Rijswijk, The Netherlands; ¹⁵RUNMC, Nijmegen, The Netherlands; ¹⁶GRCEH, Neuherberg, Germany.

* Corresponding author: George Kollias, Biomedical Sciences Research Center "Alexander Fleming", 34 Fleming St., 16672 Vari-Athens, Greece. g.kollias@fleming.gr, tel. +30 210 9656507 - fax: +30 210 9656563.

Word count: 4.411
ABSTRACT

The development of novel therapies for Rheumatoid Arthritis (RA) calls for an interactive interplay between clinical observations and studies in animal models. Given RA's complex molecular pathogenesis and highly heterogeneous clinical picture, there is currently an urgent need to dissect the multifactorial nature of the disease and to propose new strategies for preventive, early and curative treatments. Animal models of RA have been instrumental for the generation of new knowledge on disease pathophysiology and aetiology, as well as for the evaluation of novel therapeutic agents, and constitute highly successful paradigms for innovative drug development. Recent focus in RA research has shifted towards the discovery of novel biomarkers with particular emphasis on pre-symptomatic and emerging stages of human disease, and towards addressing the pathophysiological mechanisms and subsequent efficacy of intervention that underlies different disease variants. There is therefore an urgent need to integrate knowledge on human and animal models with the ultimate goal to create a comprehensive "pathogenesis map" that will guide alignment of existing and new animal models to the subset of disease that they mimic. This endeavor requires full and standardised characterization of all animal models at the genotypic, phenotypic and biomarker level exploiting recent technological developments in -omics profiling and computational biology as well as state of the art bioimaging. Shifts in the current paradigms for the mechanisms that underlie RA pathogenicity have already led to increased demand for new animal models - including humanised models - originating either from findings in human disease or from knowledge stemming from studies in experimental animals. Moreover, coordination, integration and dissemination of resources and infrastructures will be necessary to efficiently manage the plethora of data, material and know-how that will be accumulating. Outreach to the public and RA patients will be critical to increase awareness and enhance community support for innovative animal model research in Rheumatology.
This report has been prepared by the EULAR Study Group on Animal Models as a guidance document to provide recommendations on current bottlenecks and limitations in predictive RA animal modeling and to guide future directions that will enhance innovation in pre-clinical research, following a workshop organised at the EULAR House in Zurich on March 18-19, 2010.

**RHEUMATOID ARTHRITIS**

**Socio-economic impact**

Rheumatoid arthritis (RA) is a devastating, chronic inflammatory disorder that affects approximately 1% of the worldwide population, and is characterized by autoimmune reactivity and persistent active inflammation with concurrent tissue destruction[1-3]. RA is a severe burden to patients leading to disability, pain, severe impairment of quality of life, and, if not appropriately treated results in a significantly enhanced mortality[4]. Moreover, there is a striking socio-economic impact leading to direct and indirect costs of over 30 billion euros per year in Europe alone.

**Definition of disease and pathways/complexity**

It is widely accepted that RA is a systemic, autoimmune disease, with a variety of aetiopathogenic determinants acting in concert to contribute to disease initiation, progression and chronicity. These factors include genetic susceptibility, environmental stimuli, physical stress, and defective immune responses. While T- and B- cell dependent pathways have been traditionally implicated in RA development, innate immune perturbations mediated mainly by macrophages and synovial fibroblasts have also gained momentum as major orchestrators of the induction of the inflammatory cytokine milieu[2, 5, 6]. As a result of the molecular complexity that stems from the multifactorial nature of the disease, the clinical picture is highly heterogeneous with several different subsets of RA being manifested in patients. The success of specific antibody-based anti-cytokine and anti-lymphocyte therapies has underscored the importance of in depth understanding of the molecular and cellular pathways that drive RA and their contribution to disease development[7, 8].

**Unknown pathophysiologically mechanisms, hindering effective drug development**

Despite decades of intense efforts in addressing the pathophysiologic mechanisms, the pathways that drive RA causality remain unclear, hindering effective drug development. Even the best of available therapies today do not cure the disease and therapeutic goals at present are limited to the remission of symptoms. While 70% of patients with clinically established disease achieve some improvement with disease-modifying anti-rheumatic drugs (DMARDs), which currently constitute the mainstream initial treatment of RA, complete remissions are usually not observed[9]. However recent development of molecular therapies (such as anti-TNF and anti-CD20) have resulted in a new momentum and great promise for novel, more effective and safer drug development based on targeted regulation of earlier and more specific pathogenic pathways. Recently significant progress has been made to that end through successful large-scale genome-wide association studies and higher quality clinical testing of new therapies. New genetic and epidemiologic data have indicated that the disease in fact initiates many years before clinical onset, raising the demands for prevention and early diagnosis of the disease as well as for the development of therapies based on the etiologic causes rather than disease manifestations[10-12]. This new perspective underscores the significance
of reliable and predictive animal models to provide further insight into RA's early pathogenic mechanisms and to validate new therapeutic and prophylactic treatments.

**ANIMAL MODELS**

**Invaluable tools to understand disease mechanisms and validate new therapies**

Animal models of human disease provide invaluable tools to understand basic biological mechanisms, to identify and validate novel molecular pathways and targets implicated in disease pathogenesis and to screen and evaluate potential preventive and therapeutic agents. Numerous animal models for RA exist, each representing a subtype of the disease and several of those have been successfully used for target discovery and evaluation of compounds for novel therapeutic approaches. While there is no "universal model" due to RA's molecular heterogeneity and complex clinical manifestation, disease subsets are currently represented in various complementary “pathway-models”. A great advantage of using these models is that each allows modulation of a particular pathophysiological pathway, thus offering the possibility to dissect its specific contribution to disease development. The use of animal models also allows evaluation of efficacy of novel therapeutics against specific pathways. In fact both the United States Food and Drug Administration (FDA) and the European Medical Agency (EMA) guidelines require preclinical testing for new RA therapeutics on models relevant to the drug and pathways tested. Correlation and alignment of specific pathways in animal models to subsets of human disease, thus offers the unique possibility for more accurate preclinical predictions of efficacy for single or combinatorial therapeutic approaches in the clinic.

While current models do cover several aspects of the human disease, there is a longstanding need for novel, innovative studies and models that would address other aspects, such as B-cell mediated or antibody mediated mechanisms, T-cell priming of autoimmunity and regulation of chronic inflammation, macrophage or fibroblast centered chronic inflammation, osteoclast mediated destruction of the bones, breach of self tolerance, as well as disease resolution and repair. As RA can now be detected at earlier stages and severe pathology less common due to the development of biological therapies, new models are needed to reflect the new types of disease patterns that are observed in human patients.

**Higher demands on standardization and quality in experimental technology**

Clinical science has greatly benefited from standardisation of disease classification and response criteria. Moreover the set of standards for claiming significance in genetic and epidemiological studies, as well as by the high demands on clinical trials have proven to be beneficial for progress. These higher demands have also led to clinical failure of therapies and targets that have been claimed to be validated in animal models. Clearly, similarly improved quality and standardization of animal models is critically needed.

Improved genetic standards are needed in which the used inbred animal strains and crosses are genetically defined through SNP-typing and sequencing. Likewise the environmental conditions must be strictly standardized, both in terms of pathogenic, physical and behavioral environment, as well as in experimental procedures. A more precise definition of the pathogenic pathways that function in
animal models as well as the genetic and environmental context in which these operate is a key requirement, while an even more demanding task is to humanize such models by replacing genes with their human counterparts. This needs to be done with caution as the entire genomic network can be affected. Clearly, new environmental factors must also be introduced as they become identified in human disease.

Successful paradigms of animal modeling

Animal models for RA constitute highly successful paradigms for preclinical drug development based on target identification and validation in vivo and have paved the way for more focused, specific and efficient exploitation and intervention. Therapeutic approaches using biological agents developed in animal models has proven to be highly effective in clinical settings.

Standard models; CIA, CAIA and adjuvant induced models

The first animal model for RA was the Adjuvant Induced Arthritis (AIA) model in rats, which was originally induced with an intradermal injection of mycobacteria cell walls suspended in mineral oil[13]. Although commonly used, it has not proved to be an adequate model for RA (reviewed in [14]). It causes a systemic, acute inflammation with considerable suffering of the animals and which poorly reflects RA criteria. While both bacterial and oil components have been found to be arthritogenic, a new model has been developed based on pristane (the arthritogenic component discovered in mineral oil), the rat Pristane Induced Arthritis (PIA) model[14, 15], which closely mimics RA criteria, including a chronic relapsing disease course. This model is induced with synthetic and naturally occurring oil pristane, and is easy to use and highly reproducible. It is highly dependent on T cell activation and is mediated through transfer of classical MHC class II restricted T cells. It is useful for drug validation in particular for T cell related pathways leading to arthritis.

The most commonly used arthritis model is type II Collagen Induced Arthritis (CIA)[16-18]. It is one of several models that can be induced through immunization with various cartilage proteins in mice, rats and monkeys, breaking tolerance and directing an immune mediated inflammatory attack on the joints. The most commonly used variant of CIA is through immunization with type II collagen (CII) emulsified in complete Freund’s adjuvant using the high responder DBA/1 mouse. Although easy to use it has clear drawbacks as a model for RA; it is an acute model that fulfills only a few of the RA classification criteria. It is also highly variable dependent on the quality of the used CII and environmental factors such as grouping stress that the DBA/1 is particularly sensitive for and which can lead to spontaneous development of arthropathy[19]. Better alternative variants of CIA include the use of different mouse strains that allow a more RA similar disease course, such as F1 strains including the C57Bl background together with a susceptible MHC class II q haplotype. For example the F1 (B10.QxBalb/c) develops a chronic arthritis induced with CII emulsified in mineral oil only without the use of mycobacteria. The acute CIA models, as in DBA/1 strain, are dependent on arthritogenic antibodies and a more defined model based on the induction of arthritis using monoclonal anti-CII antibodies is widely used, the Collagen Antibody Induced Arthritis (CAIA) model[20, 21]. It is a well controlled and reproducible model that may answer questions related to the antibody induced effector phase in arthritis.
CIA models in non-human primates have also been developed in response to the need of a relevant preclinical RA model in which new therapeutic agents that are inactive in lower species, monoclonal antibodies or cytokines (antagonists) for example, can be tested.

**Human TNF transgenic models (Tg-huTNF)**

Tumor necrosis factor (TNF) is a key player in the development of RA pathogenesis[22-28]. The pathogenic potential of TNF and the reversal of disease progression by anti-TNF antibodies has been originally demonstrated in the Tg-huTNF transgenic mouse model[25]. This model is characterized by deregulated expression of human TNF that leads to the development of chronic, erosive and symmetric polyarthritis, with histological characteristics resembling human RA. Penetration in this model is 100% and disease progression is highly homogeneous, with synovitis and cartilage destruction occurring even in the absence of an adaptive immune system (e.g. in RAG-/- crosses) and adaptive responses regulating mainly bone destruction[29]. The Tg-hTNF mice represent a valuable model restricted to TNF-driven mechanisms of disease and as such it may not be suitable for the assessment of upstream or TNF-independent pathways. Recently, more specific TNF-driven models have been generated by restricting TNFR1 expression in synovial fibroblasts[5].

**Other arthritis models**

Several additional models for RA have also been developed, including transgenic models such as the KRN arthritis (transgenic TCR recognizing a peptide from a ubiquitously expressed glycolytic enzyme), SKG (altered TCR-signalling), GP130 (altered IL-6R-signalling) and IL-1 (altered IL-1 signalling) models and immune complex models, such as the KRN serum transfer model. In similarity with the older adjuvant arthritis models these genetically altered models display pathogenic adaptive immune responses without any known specific recognition of joint antigens, challenging the idea that recognition of autoantigens specifically expressed in the joints is necessary for the induction of autoimmune arthritis. In the KRN model the antigen is defined by the transgenic expression of a TCR recognizing glucose 6 phophoisomerase, a protein occurring in all cells. The arthritis is however mediated by serum antibodies recognizing the G6PI on the cartilage surface. The SKG model is a spontaneous mutation in the TCR signaling adapter molecule ZAP70 and when the mice are induced with specific adjuvants they develop severe autoimmune arthritis. Several of these models have only been developed recently and further studies and comparative analyses are awaited to determine their predictive and translational capacity, as well as their alignment to human disease subsets[30]. A comprehensive overview of RA animal models can be found in the recent review by van den Berg[31].

**Need for new models/pathways/biomarkers**

Animal models have been instrumental for the development of novel biological therapies for RA, and can accommodate shifts in the current paradigms for the molecular mechanisms on which RA pathogenicity is based. In the future the increasing demands on new animal models will include:

1. **Development of models that reflect pathways revealed by findings in human disease**

These could be new genetic polymorphisms, but also environmental factors such as the new discoveries of an interaction between smoking, production of ACPA, MHC class II association and
arthritis[12]. The animal models need to be well controlled for studies of the specific genetic and environmental factor as well as the contextual i.e. the real genetic and physical standardized environment of the mouse. Clearly, the human disease is heterogeneous and needs to be studied with focus on specific pathways. For example, treatments based on TNF blockers or IL-6 inhibitors while highly effective in some cases, do not work on all patients. It is therefore necessary to develop models that will represent the various subtypes in RA.

2. New animal models based on knowledge originating in experimental animals

Particular emphasis needs to be given on the mechanisms driving the earliest pathogenic steps as well as the mechanisms perpetuating chronic inflammation. This requires development of genetically modified, mutated and congenic models in which precise disease mechanisms can be studied in a defined context. It will also require “omics” approaches on the mouse, similar to those done in humans, i.e. analysis of genetics, epigenetics, proteomics and glycomics in a hypothesis-free way in order to identify the naturally selected forces paving the disease pathways. Standardisation of these models is a prerequisite for them to be useful for validation experiments testing new preventive and therapeutic strategies.

3. Humanised animal models

Use of humanized animal models that either have a human hematopoietic system or in which key genetic components have been replaced by their human counterparts is also of major importance[32]. For example, transgenic mice expressing HLA class II molecules have been used to show that polymorphism of HLA class II genes determine the predisposition to rheumatoid / inflammatory arthritis[33]. Humanization can enhance a model’s predictive value in preclinical efficacy evaluations of compounds directed against the human target, but can also provide more accurate predictions for the toxicity and safety of tested pharmaceuticals before clinical trials. A potential limitation is that introduction of human genes into the mouse may result in unexpected, non-physiological interactions with the mouse genome.

So far, there are no humanized mouse models of RA in terms of reconstitution of the immune system. The SCID mouse, that currently is the only humanized mouse model for RA, consists in engrafting human cartilage together with RA synovial fibroblasts (RA-SF) subcutaneously into severe combined immunodeficient (SCID) mice[34]. This SCID model allows the evaluation of potential drugs on the aggressive behavior of RA-SF, a key player in joint destruction, by measuring cartilage invasion. By transposing this RA model into human immune system mice, the role for human innate and adaptive lymphocytes in the pathophysiology of RA could be further dissected.

4. Novel molecular biomarkers

The development of new animal models through the discovery of novel molecular targets and pathogenic pathways, will also provide novel predictions for biomarkers that may also be of relevance to human disease. There is currently a high demand for biomarkers that allow:

- Better correlation of animal models to human disease subsets
- Facilitation of early disease diagnosis and prognosis
- More precise and reliable disease staging
- Accurate prediction of treatment responses
Facilitating the alignment of animal models to human disease using emerging technologies

A first bottleneck in efficient exploitation of animal models for drug discovery is the lack of appropriate alignment of animal models to various subsets of the human disease. RA manifestation depends on a multitude of genetic and environmental factors that lead to differential initiation, progression, chronicity and resolution events. Disease subsets are defined by response to particular therapies (for example anti-TNF responders or non-responders), mechanistic hypotheses underlying disease pathogenesis, and molecular biomarkers, such as the presence or absence of antibodies to citrullinated protein antigen (ACPA), or rheumatoid factors (RF), which often precede disease onset by several years and is in fact closely linked to particular MHC class II alleles. The predictive value of such markers, together with genetic factors, such as MHC class II genes, and environmental factors such as smoking, are likely to change dramatically the therapeutic strategies for RA, from symptom management towards prevention and cure.

It is therefore necessary to create a comprehensive "pathogenesis map" outlining the current knowledge on human RA, on which animal models will be aligned according to the specific aspect/subset of the disease that each of them reflects, a task that is both challenging and ambitious. This will help to define the criteria for the selection of the most appropriate animal model for a given question or pathway and will highlight areas not covered by the currently available models. This endeavor requires full and standardised characterization of each model at the genotypic, phenotypic and biomarker level. Comprehensive phenotyping should exploit recent technological developments in large scale, “-omic” profiling, including chromatin structure, epigenetic, miRNA, transcriptional, post-transcriptional and proteomic analysis, as well as state-of-the art bioimaging of implicated tissues and cell types. The association of phenotypes to molecular profiles (gene, protein and metabolite expression) offers an invaluable tool not only for unveiling pathogenic mechanisms, pathways and targets, but also for discovering predictive biomarkers for diagnosis and treatment.

RECOMMENDATIONS

Integration of resources and generation of new ones (technological and infrastructure)

Phenotyping and mutagenesis resources

Particular effort must be dedicated to the integration of available resources and generation of new ones (technological as well as infrastructure ones). Investigator driven, bottom up approaches, should be coordinated and balanced with large collaborative or infrastructure based projects (such as INFRAFRONTIER, EMMA, EUMODIC and EUCOMM), including secondary phenotyping, archiving and distribution projects, standardized mouse cohorts, and strain repositories, to facilitate innovative scientific advancements in the field. New models should be designed such that they are easily genetically manipulated (e.g. cre-lox, optogenetics etc) and amenable to technologies such as state-of-the-art imaging for comprehensive phenotyping (i.e. they should be compatible with reporter strains or have reporters expressed as routine). Given the large amount of existing and anticipated
models for RA, it is important to support and coordinate such efforts for more efficient exploitation of results in a pan-European level.

**Large-scale profiling and metadata integration across platforms and models**

While several fragmented resources exist in Europe containing large amounts of information including –omics data at a “system” level, it is at the moment not possible to correlate and integrate them. It is therefore of imperative importance to create relational databases in which phenotypic, molecular, profile and clinical data from animal models and patients will be deposited and processed in a systematic and hierarchical way that will allow their computational integration, synthesis and exploitation. These resources should be designed such that they allow rapid and easy access to experimental data in a dynamic and fully searchable way and ensuring interoperability with other databases. Preferably raw data of published experiments should be made available as supplementary information and published on journal servers, publicly founded servers or authors’ servers to allow the development of in silico modeling of in vivo data. In the future such data can be compared with historical published data to allow new findings overlooked by single investigators.

**New environmentally influenced models and standardised biobanks**

In order to be able to compare and align animal models it is instrumental to have standardized resources for inbred strains through dedicated facilities such as the Jackson laboratories, and frozen embryos of specific strains available at several centers. Rapid expansion and integration of standardized information in silico, such as genome sequences, SNP markers, transcriptome analysis, available strains in various laboratories etc. is also of vital importance. Genetic and environmental information on each used strain in published experiments must be available. It is no longer enough to rely on historic designations and descriptions of genetic origin and environmental conditions. Used strains must be quality-controlled by genotyping or sequenced and the environmental conditions of importance for the experiments need to be strictly documented. While such resources already exist, they are at present limited and fragmented and they need to be integrated and expanded.

**Standard operating procedures and quality requirements for validation and discovery testing**

It is of utmost importance to introduce standard operating procedures for the most commonly used animal models. Models should be scientifically well defined, representing different disease pathways relevant for RA, highly reproducible, with no commercial restrictions and readily available.

Strict quality criteria for operating and evaluating animal models must be set. This will not be done in detail here but awaits a consensus protocol for each model. It is however important to introduce some urgent and obvious minimal requirements:

1) **Use of genetically defined mice.** The rapid production of new genetically modified strains has led to the introduction of genetic and experimental artifacts. To raise the quality we need to have defined backgrounds, and when possible use a standard background. Furthermore, to prevent both genetic and environmental confounding it is highly recommended to use littermate experiments, blinded experiments and mixing groups in cages. Preferably these should be genetically defined by marker analysis of the actual used mice or from the provider. The use of a standard genetic background such as C57BL/6N (which is most commonly used in ES cell derived mice[35] and suitable
for most basic research applications) is important to standardize and compare results. It is however essential to point out that models of arthritis should not be limited to a single genetic background, as different strains of mice confer differential susceptibility to different types of arthritis and provide the variability that is required for comparisons with the human disease. New types of genetic modification and conditional mutagenesis technologies that allow better genetic control should in any case be preferred.

2) **Defined environmental conditions.** New demands on caging and environmental enrichment have often led to higher variability both within experiments and in particular between different laboratories. To prevent this, experiments must be performed under strictly defined and standardized experimental conditions such as defined housing conditions, IVF/open, light, temperature, humidity, caging environment (standard bedding, with highly standardised environmental enrichment only when it does not interfere with the variability of the disease course) and pathogen standards (if not fulfilling Felasa criteria, the specific pathogens that occur need to be indicated). Defined environmental conditions will facilitate comparison of results and help eliminate phenotypic heterogeneities observed when transferring mice between facilities.

3) **Sufficient numbers of animals per group/experiment.** Despite the cost of animal experiments, and governmental regulations, that have increased dramatically, the number of animals that is used in each experiment must always be determined by power calculation to allow for statistical significance, and cannot be compromised. Using less than the recommended minimum amount of mice, may lead to less statistical power, and less reproducible results, which would therefore be less useful for translation to clinical studies. Biological variation needs to be clearly indicated so that the reader can estimate the statistical power of the experiment.

4) **Use of proper controls.** This includes control groups identical except what is investigated (gene or treatment), i.e. the use of littermates, sham operations and solvent treatments are required. Experiments need to be balanced for sex and age and control/probands must be mixed in cages to avoid cage effects.

5) **Defined experimental procedures.** We need to exactly define and describe the purity and physical state of antigen/adjuvant that is injected, the amount, as well as the route of injection.

6) **Standardized evaluation.** Evaluation of disease needs to be done without knowledge of the group identity ("blind" evaluation). It is also a necessity that all performed experiments are shown and taken into the statistical evaluation to fully document the biological variation as all animal models and their underlying traits are quantitative variables.

**In silico modeling for predictions of diagnosis, prognosis and therapy**

In silico modeling and computational simulations provide a valuable tool throughout the drug development process, from early target and hit identification to clinical trials. Modeling can facilitate the translation of preclinical results into reliable predictions for drug efficacy and safety in the clinic, by reducing the timeframe and cost of the discovery pipeline, and increasing the success rate of clinical trials. Dynamic mathematical, mechanistic models (such as "virtual animal models") that are built through integration of genomic, proteomic, biochemical, physiological and environmental data, can help simulate physiology and disease and allow predictions of clinical responses to potential therapeutics. In silico modeling can also significantly reduce the number of laboratory animals used
in preclinical evaluation of drugs, a notion fully compatible with the 3R concept that governs all preclinical testing in animal models. Nonetheless, computational simulation is unable to deal with unknown factors and layers of regulation and requires deep knowledge of physiological and pathophysiological processes, as well as high quality and quantity data that can only be obtained from wet-lab biological experiments.

**Ethical considerations for the use of animal models**

While at the moment there is no alternative to the use of animal models for preclinical evaluation of RA therapeutics, all research in this area must be conducted under the principles of "**reduction, replacement and refinement**" of animal use in experimental protocols. Focused and standardised procedures will undoubtedly lead to the reduction and refinement in the use of animal models and will diminish the 'harm to benefit' ratio in the justification of animal use in preclinical research. Especially in the field of Rheumatology, the use of animal models has paved the way for the development of novel therapeutics resulting in a marked improvement of RA patients' quality of life. Continued dynamic interactions between scientists and society will be necessary in order to increase awareness of the usefulness of animal modeling in curing disease and to enhance support for innovative translational research in the field.

**ACKNOWLEDGEMENTS**

The authors wish to thank the European League Against Rheumatism (EULAR) for supporting and funding the EULAR Study Group on Animal Models, as well as members of the Autocure (LSHB-CT-2006-018661), Masterswitch (HEALTH-F2-2008-223404) and MUGEN (LSHG-CT-2005-005203) consortia for initiating this effort.

The authors have no competing interests.

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non-exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in Annals of the Rheumatic Diseases and any other BMJPLG products to exploit all subsidiary rights, as set out in our licence ([http://ARD.bmjjournals.com/ifora/licence.pdf](http://ARD.bmjjournals.com/ifora/licence.pdf)).

**REFERENCES**


