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Animal models for arthritis: innovative tools for prevention and treatment

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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.

RHEUMATOID ARTHRITIS

Socioeconomic impact

Rheumatoid arthritis (RA) is a devastating chronic inflammatory disorder that affects approximately 1% of the worldwide population and is characterised by autoimmune reactivity and persistent active inflammation with concurrent tissue destruction.^{1–3} It is a severe burden to patients leading to disability, pain, impaired quality of life and, if not appropriately treated, results in significantly enhanced mortality.⁴ Moreover, there is a striking socioeconomic impact leading to direct and indirect costs of over €30 billion 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 the development of RA, 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 anticytokine and antilymphocyte therapies has underscored the importance of an in-depth understanding of the molecular and cellular pathways that drive RA and their contribution to disease development.^{7 8}

Unknown pathophysiological mechanisms hindering effective drug development

Despite decades of intense efforts in addressing the pathophysiological 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 antirheumatic drugs 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-tumour necrosis factor (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 epidemiological 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 aetiological causes rather than disease manifestations.^{10–12} This new perspective underscores the significance of reliable and predictive animal models to provide further insight into the early pathogenic mechanisms of RA 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 the basic biological mechanisms, to identify and validate novel molecular pathways and targets implicated in the pathogenesis of the disease 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 these have been successfully used for target discovery and evaluation of compounds for novel therapeutic approaches. While there is no 'universal model' due to the molecular heterogeneity and complex clinical manifestation of RA, 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 US Food and Drug Administration 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 aspects such as B cell-mediated or antibody-mediated mechanisms, T cell priming of autoimmunity and regulation of chronic inflammation, macrophage- or fibroblast-centred chronic inflammation, osteoclast-mediated destruction of bones, breach of self-tolerance, as well as disease resolution and repair. As RA can now be detected at earlier stages and severe pathology is less common owing 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 standardisation and quality in experimental technology

Clinical science has benefited greatly from standardisation of disease classification and response criteria. Moreover, the set of standards for claiming significance in genetic and epidemiological studies as well as the high demands on clinical trials have been 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 standardisation of animal models is critically needed.

Improved genetic standards are needed in which the inbred animal strains and crosses used are genetically defined through single nucleotide polymorphism (SNP) typing and sequencing. Likewise, the environmental conditions must be strictly standardised in terms of pathogenic, physical and behavioural environment as well as in experimental procedures. A more precise definition of the pathogenic pathways that function in animal models and the genetic and environmental context in which these operate is a key requirement, while an even more

demanding task is to humanise 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 disease determinants must also be introduced as they become identified in human disease.

Successful paradigms of animal modelling

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 have proved to be highly effective in clinical settings.

Standard models: collagen-induced arthritis, collagen antibody-induced arthritis and adjuvant-induced models

The first animal model for RA was the adjuvant-induced arthritis 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 Holmdahl *et al*).¹⁴ It causes a systemic acute inflammation with considerable suffering of the animals and 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 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 major histocompatibility complex (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 immunisation 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 immunisation 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, being dependent on the quality of the CII used and environmental factors such as grouping stress to which the DBA/1 mouse is particularly sensitive 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 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.Q × Balb/c) develops a chronic arthritis induced with CII emulsified in mineral oil only without the use of mycobacteria. The acute CIA models, as in the 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 model.^{20 21} It is a well-controlled and reproducible model that may provide answers related to the antibody-induced effector phase in arthritis.

CIA models in non-human primates have also been developed in response to the need for 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)

TNF is a key player in the development of the pathogenesis of RA.^{22–28} The pathogenic potential of TNF and the reversal of disease progression by anti-TNF antibodies was originally demonstrated in the Tg-huTNF transgenic mouse model.²⁵ This model is characterised by deregulated expression of human TNF leading to the development of chronic, erosive and symmetrical polyarthritis with histological characteristics resembling human RA. Penetrance 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 (eg, in RAG^{−/−} crosses)²⁸ and adaptive responses regulating mainly bone destruction.²⁹ The Tg-huTNF mouse represents 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 T cell receptor (TCR) recognising a peptide from an ubiquitously expressed glycolytic enzyme), SKG (altered TCR signalling), GP130 (altered interleukin 6R (IL-6R) signalling) and IL-1 (altered IL-1 signalling) models and immune complex models such as the KRN serum transfer model. Similar to 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. For example, in the KRN model the antigen is defined by the transgenic expression of a TCR recognising glucose-6-phosphoisomerase (G6PI), a protein that occurs in all cells. However, the arthritis is mediated by serum antibodies that recognise the G6PI on the cartilage surface. The SKG model is a spontaneous mutation in the TCR signalling adapter molecule ZAP70 and, when the mice are induced with specific adjuvants, they develop severe autoimmune arthritis. Several of these models have only recently been developed 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 in 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 future, the increasing demands on new animal models will include:

- ▶ Development of models that reflect pathways revealed by findings in human disease.
- ▶ New animal models based on knowledge originating in experimental animals.
- ▶ Humanised animal models.
- ▶ Novel molecular biomarkers.

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

New models originating from novel findings in humans could be based on new genetic polymorphisms, but also on environmental factors such as the newly discovered interactions between smoking, production of antibodies to citrullinated protein

antigen (ACPA), MHC class II association and arthritis.¹² The animal models need to be well-controlled for specific genetic and environmental factors as well as for the contextual—that is, the real genetic and physical standardised—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.

New animal models based on knowledge originating in experimental animals

Particular emphasis needs to be given to 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 in humans—that is, 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.

Humanised animal models

Use of humanised animal models that either have a human haematopoietic 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 human leucocyte antigen (HLA) class II molecules have been used to show that polymorphism of HLA class II genes determines the predisposition to rheumatoid/inflammatory arthritis.³³ Humanisation 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 toxicity and safety of tested drugs 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.

To date there are no humanised mouse models of RA in terms of reconstitution of the immune system. The severe combined immunodeficient (SCID) mouse model, which is currently the only humanised model for RA, consists in engrafting human cartilage together with RA synovial fibroblasts (RA-SF) subcutaneously into SCID mice.³⁴ This SCID model allows the evaluation of potential drugs on the aggressive behaviour 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 of human innate and adaptive lymphocytes in the pathophysiology of RA can be further analysed.

Novel molecular biomarkers

The development of new animal models through the discovery of novel molecular targets and pathogenic pathways will 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 diagnosis and prognosis of disease.
- ▶ 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 the response to particular therapies (eg, anti-TNF responders or non-responders), mechanistic hypotheses underlying disease pathogenesis and molecular biomarkers such as the presence or absence of ACPA or rheumatoid factors, 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 endeavour requires full and standardised characterisation 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 for unveiling pathogenic mechanisms, pathways and targets and 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 the generation of new ones (technological as well as infrastructure). 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, standardised mouse cohorts and strain repositories to facilitate innovative scientific advances in the field. New models should be designed so that they are easily manipulated genetically (eg, cre-lox, optogenetics) and amenable to technologies such as state of the art imaging for comprehensive phenotyping (ie, 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 at a pan-European level.

Large-scale profiling and metadata integration across platforms and models

While there are several fragmented resources in Europe containing large amounts of information including -omics data at a 'system' level, it is not currently possible to correlate and integrate them. It is therefore important to create relational databases in

which phenotypic, molecular profiling 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 to allow rapid and easy access to experimental data in a dynamic and fully searchable way and to ensure 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* modelling 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 necessary to have standardised resources for inbred strains through dedicated facilities such as the Jackson laboratories and frozen embryos of specific strains available at several centres. Rapid expansion and integration of standardised information *in silico*—such as genome sequences, SNP markers, transcriptome analysis, available strains in various laboratories and so on—is also of vital importance. Genetic and environmental information on each strain used in published experiments must be available. It is no longer enough to rely on historical 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:

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, to use a standard background. Furthermore, to prevent both genetic and environmental confounding, it is highly recommended to use littermate and blinded experiments, as well as to mix groups in cages. Preferably, these should be genetically defined by marker analysis of the actual mice used or by the provider. The use of a standard genetic background such as C57Bl/6N (which is most commonly used in embryonic stem cell-derived mice³⁵ and is suitable for most basic research applications) is important to standardise and compare results. It should, however, be pointed 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.

Defined environmental conditions

New demands on caging and environmental enrichment have often led to higher variability within experiments and, in particular, between different laboratories. To prevent this, experiments must be performed under strictly defined and standardised 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.

Sufficient numbers of animals per group/experiment

Despite the cost of animal experiments and dramatically increased governmental regulations, the number of animals used in each experiment must always be determined by power calculation to allow for statistical significance and cannot be compromised. Using fewer than the recommended minimum number 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.

Use of proper controls

This includes control groups that are identical except for what is being investigated (gene or treatment)—The use of littermates, sham operations and solvent treatments are strictly required. Experiments need to be balanced for sex and age and control/probands must be mixed in cages to avoid cage effects.

Defined experimental procedures

The purity and physical state of antigen/adjuvant that is injected, the amount as well as the route of injection, must be strictly defined and described.

Standardised evaluation

Evaluation of disease needs to be done without knowledge of the group's identity ('blind' evaluation). It is also necessary that all performed experiments are shown and taken into account during the statistical evaluation to fully document the biological variation as all animal models and their underlying traits are quantitative variables.

In silico modelling for predictions of diagnosis, prognosis and therapy

In silico modelling and computational simulations provide a valuable tool throughout the drug development process, from early target and hit identification to clinical trials. Modelling 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 to simulate physiology and disease and allow predictions of clinical responses to potential therapeutics. In silico modelling 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-laboratory 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 of use of animal models and will diminish the 'harm to benefit' ratio in the justification of animal use in preclinical research. In the field of rheumatology in particular, the use of animal models has paved the way for the development of novel therapeutics resulting in a marked improvement of in the quality of life of patients with RA. Continued dynamic interactions between scientists and society will be necessary in order to increase awareness of the usefulness of animal modelling in curing disease and to enhance support for innovative translational research in the field.

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