Evaluating the validity of animal models for research into therapies for immune-based disorders

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The last few decades of the 20th century have shown an intensified search for safer and more effective medications against chronic diseases that burden ageing societies of the western world. The impressive development of biotechnological production techniques has greatly facilitated the pharmaceutical development of relatively non-toxic biological molecules. However, despite the huge investments, only a few effective therapies for immune-based diseases have reached the clinic. In this article we use examples from monoclonal antibody trials to discuss the validity and predictive strength of the animal models currently used for the development of effective therapies.

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▼ Many clinical disorders in the western world are caused by unwanted activities of the immune system that fall into the categories of: (1) allergy and asthma; (2) autoimmune diseases; and (3) the rejection of transplanted organs. The high incidence of these disorders and the high cost to society has stimulated the drug industry to invest heavily in the development of safe and effective therapies. In particular, the search for biological molecules acting specifically to overcome the considerable side effects of non-specifically acting anti-inflammatory and immunosuppressive drugs has increased exponentially [1]. Biotechnology offers a broad arsenal of engineering techniques to produce such specific bioactive drugs in sufficient quantity and of high pharmaceutical quality. Shortly after the first publication on the principle of monoclonal antibody (mAb) production in 1975 [2], which was awarded the 1984 Nobel Prize for Medicine, the enormous potential of mAb technology for diagnosis of disease [3] and development of highly selective immunotherapy was recognized [4]. MAbs were first used therapeutically for the elimination of leukocyte malignancies and of the immune cells that cause the rejection of transplanted kidney [5,6]. At that time treatment of autoimmune diseases was not yet considered feasible, although beneficial effects of treatment with anti-MHC class II (anti-Ia) antibodies in mouse models of autoimmune diseases, such as multiple sclerosis (MS) or systemic lupus erythematosus (SLE), had been reported [7,8].

The lack of trust in mAbs for human diseases might have been due to the unexpected toxicity of some mAbs when tested in higher species. For example, anti-DR mAbs were found to cause significant toxicity in cynomolgus macaques, a side effect that was not common in mouse models, probably because of the different expression of MHC class II molecules between rodents and primates [9]. A consequence of relying mainly on rodent studies for so-called 'preclinical' testing of therapeutic antibodies was that the first-treated patients suffered unexpected side effects due to cytokine release syndrome and many of the dosing problems required re-evaluation in post-registration studies. It was not until Maini and Feldmann first showed that prolonged administration of a mAb directed against TNF-α was safe and effective in RA patients that mAb therapy for chronic diseases in humans was considered a real possibility [10]. The likelihood that similar strategies for other immune-based diseases will be used and be yet more successful, has been due to the development of humanized antibodies. However, due to the great species difference, safety tests of these mAbs are not possible in lower species such as rodents. Animals more closely related to humans, such as non-human primates (NHP), should be used in this case.

1359-6446/04/$ – see front matter PII: S1359-6446(04)03112-5

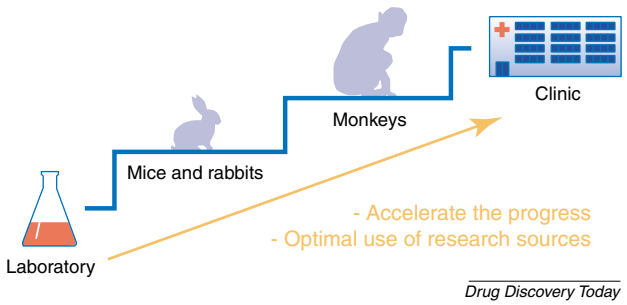

Figure 1. Preclinical testing of biological drugs. Many therapeutic principles are based on the observation that certain reagents exert specific effects on immune cells. Proof of a therapeutic principle is usually obtained in disease models in lower species. When a promising effect is observed, new molecules have to be generated that recognize the equivalent target molecules in humans. Because of their outbred nature and immunological proximity to humans, non-human primates offer unique disease models to test whether the therapeutic principle holds in a higher species and whether the redesigned biologicals are as safe and effective as the parent molecule.

The few cases where trials with biological drugs were successful contrast with a long list of failures where promising effects in animal models could not be reproduced in patients (see following paragraphs and [11]). Were expectations too high and have the huge investments by the biotechnology industry been a waste of money? We do not think so, but we have to consider why, of the many treatment protocols that were effective in mice and rats with experimental autoimmune diseases, so few have reached the clinic. It is, therefore, perfectly reasonable to question whether the currently used preclinical animal models are sufficiently valid for, and predictive of, the safety and efficacy of biological therapies in humans.

Requirements for a valid preclinical model

Animal models are used in the drug development process for the identification of targets for therapeutic intervention and to provide proof of a therapeutic principle. The most-widely used animal models use inbred strains of mice and rats, whether testing either non-biological or biological drugs. When a reagent shows promising effects in initial screening, it might be entered into a preclinical testing phase. This is important, as it could eventually reveal whether the therapeutic principle established using rodent models is valid for humans. To ensure a reliable prediction of the safety and efficacy of a new therapy in humans, the choice of valid preclinical animal models is crucial (Figure 1).

A first selection criterion is the target-specificity of the animal model. In contrast to classical immunosuppressive drugs, biological drugs such as mAbs and soluble cytokine or chemokine receptors are made highly specific for the target molecule. With these 'magic bullets', cells or soluble molecules can be specifically eliminated. Obviously, the target molecule in the chosen 'preclinical' animal model should be sufficiently homologous to the equivalent structure in patients to be recognized by the new drug. Moreover, the target molecule should have a similar expression pattern and pharmaco-dynamic properties as in the human disease. In many cases, rats and mice are too distant from man to fulfill these requirements. However, a model in engineered mice, such as humanized SCID mice or mice in which the human target molecule is expressed as a transgene under the control of an organ-specific promoter, can provide useful alternatives.

A second criterion is safety assessment. Ideally the model should predict adverse side-effects in patients. Biological drugs are based on molecules produced by the body itself and are often thought to be relatively non-toxic. However, two frequently encountered hazardous syndromes in preclinical trials with biological drugs are 'anaphylactic shock' and 'cytokine release syndrome.' Anaphylaxis occurs when drugs form immune complexes with pre-existing antibodies, causing massive activation of the complement cascade. The sudden release of vasoactive complement factors can cause clinical shock. In cytokine release syndrome, antibodies binding to cell surface molecules cause extensive release of cytokines by the targeted cells, in some cases inducing a life-threatening shock reaction. This was observed with the T-cell specific mAb OKT3, developed for the prevention of graft-rejection. Although OKT3 could not be extensively tested in a preclinical transplantation model in non-human primates (NHP) due to lack of cross reactivity of the OKT3 mAb with the CD3 molecule of macaques, the antibody did cross-react with CD3 of chimpanzees; however, a safety assessment was not performed.

A unique feature of biological molecules, that is not usually encountered with small molecular drugs, is the high immunogenicity, that is, the induction of a neutralizing immune response leading to a reduced efficacy of the drug. It is difficult to prevent this as the immune system senses minor differences from self, for example, by the mere substitution of a few amino acids or slight changes in post-translational modification. Immunogenicity of biological therapeutics is not only a major hurdle in the development of effective biological molecules for treatment of chronic diseases, but is also a safety concern. For example, apart from hypersensitivity problems due to immune complex formation, neutralizing antibodies raised against a certain recombinant cytokine or chemokine makes it ineffective [12]. Remarkably, the potential consequences of

the long-term disturbance of a tightly regulated network of endogenous cytokines receives little attention [13]. Whereas the problem of the immunogenicity of mAbs has now been reduced significantly with the use of fully humanized antibodies, this is still a problem with recombinant proteins [14].

It is poorly understood why so many therapies that work well in rodents have been ineffective in clinical trials. In several cases, even unforeseen detrimental effects have been reported, such as in trials with anti-TNFα, or altered peptide ligands in multiple sclerosis patients. Thus, the central question is how representative an experimental disease model in a 12-week-old, inbred and pathogen-free, laboratory mouse is for a heterogeneous population of patients that have a long history of infections that have been suggested to shape the immune repertoire [15]. It requires little imagination to realize that the substantial immunological difference between mice (and most likely also other rodents) and man has implications for the validity and predictability of preclinical disease models in these species [16,17]. It is, therefore, highly remarkable that, in transplantation research, preclinical tests in non-human primates are regarded as an essential bridge between rodent models and patients [16], whereas those developing therapies for chronic diseases rely mainly on classical inbred rodent models, although excellent disease models exist for several of such diseases.

Besides general requirements to a valid preclinical disease model there are also specific requirements related to the disease that is to be targeted. Specific issues in preclinical therapy development for graft rejection and chronic diseases will be discussed.

Table 1. List of drugs tested in non-human primates before clinical application

Drug	Working mechanism	Refs
ATG	T-cell depletion	[47]
CsA (cyclosporine) Prograft (tracolimus, FK506)	Calcineurin inhibitors	[48]
Rapamycine (sirolimus) SDZ-RAD (everolimus)	TOR inhibitors	[49]
CellCept (mycophenolate mofetil)	Inhibition guanosine nucleotides	
Zenapax (daclizumab) Simulect (basiliximab)	Anti-CD25 antibody	[50]

Preclinical models for immunotherapy in transplantation

Current problems in transplantation are the shortage of donor organs, adverse effect of prolonged immunosuppression and chronic allograft pathology. Organ shortage could be solved by using artificial organs or donor organs from pigs. The feasibility of pig organ transplantation should be tested in NHP, which combine a sufficient size with human-like organ physiology and the immunological characteristics. Problems associated with prolonged immunosuppression and chronic allograft pathology can be solved by induction of graft-specific tolerance. The *ex vivo* examination of human allo-responses shows that, for prevention of graft rejection, cellular as well as humoral immune responses need to be controlled. One way of achieving stable tolerance is to create donor hemopoietic cell chimerism by recipient pretreatment with donor cells after a myeloablative treatment regimen in the recipient [18]. This approach can be performed relatively easily in rodent models by the transplantation of donor bone marrow after whole-body irradiation or chemotherapy. Such hemopoietic chimeras accept donor grafts without the need for immunosuppression. Suitable myeloablative regimens for clinical application are best investigated in NHP. Not only is the physiological response to the myeloablative regimen more similar to man, but also the conditioning drugs (such as ATG) cross-react only with closely related species [19]. Most clinicians hesitate using this approach in their patients, as the severe conditioning regimen will only be acceptable in a few selected cases.

Alternatively, the recipient's immune system can be deceived at the time of transplantation to establish permanent suppression of the allo-specific immune reaction to the donor. Of 12 effective protocols for allo-specific tolerance induction in rodents, only one was successful in man, namely induction of donor chimerism, and three were only partially effective [16]. Explanations for this are the higher genetic diversity and immunological maturity of humans compared with rodents. Indeed, tolerance induction in mice becomes more difficult when they have experienced more viral infections, leading to a more extensive repertoire of memory cells that could prevent tolerance induction [15]. Thus, tolerance protocols should be evaluated in animals that resemble man more closely than naïve, inbred mice. Preclinical tests of new immunosuppressive drugs in NHP have, up to now, been more predictive (see Table 1). The polyclonal T-cell-specific antibody ATG was the first biological drug developed for transplant patients and still has a widespread use as immunosuppressive [20]. For many years, the regulatory authorities requested

Table 2. Evaluation of antibodies in non-human primate transplantation models compared with rodents and clinical application

Drug	Outcome in rodents	Outcome in NHP	Clinical trials
Overview	Review [51]	Review [22]	Review [52]
Anti-CD4	Tolerance [53,54]	Graft prolongation [55–57]	Stopped
Anti-CD154	Tolerance [58]	Near tolerance [59]	Stopped
Anti-B7-1,2	Not tested	Graft prolongation [60]	Started
CTLA4-Ig (LEA29Y)	Tolerance [61]	Graft prolongation [62]	Ongoing
Anti-CD40	Not tested	Graft prolongation [62,63]	Ongoing (Crohn's disease)
Anti-MHC Ab	Graft prolongation [64]	Graft prolongation (Jonker, M. unpublished data)	Development stopped
Anti-CD45 RB	Tolerance [65]	Graft prolongation [65]	Not tested

NHP testing before ATG batch release. ATG had a follow up in the 1980s with the anti-CD3 antibody OKT3 [21]. As discussed previously, the safety of OKT3 could not be tested in macaques due to insufficient cross reactivity and the first OKT3-treated patients suffered unexpected cytokine release syndrome. Campath-1H (anti-CD52) could represent an alternative for anti-CD3 and ATG, but safety and efficacy trials again could not be tested in macaques, as the antibody cross reacts with the monkey red blood cells [22]. However, as the antibody has been registered for lymphoma therapy in humans, extensive clinical studies were initiated.

Although these T-cell specific antibodies are effective in humans, their broad mode of action does not allow induction of subtle T-cell regulatory networks. Antibodies blocking the co-stimulation of T-cells are considered more useful. Several promising new biological reagents, using other mechanisms to induce specific suppression of anti-graft responses, have been listed in Table 2. Well-designed studies in NHP of mono- or combination therapies will be needed to select the optimal treatment protocol.

An example of an intelligent protocol developed in NHP is the combination of a short period of anti-CD3-toxin, followed by 15-deoxyspergualine (DSG) preventing acute rejection. This treatment protocol blocks the direct pathway of immune stimulation, preventing acute rejection by T-cell depletion, as well as the indirect pathway, abolishing chronic rejection by prevention of dendritic cell maturation in NHP [23]. In the current situation, where with classical immunosuppression ~90% of transplanted organs survive the first critical year post transplantation, the effectivity of new therapies should be proven in a valid non-human primate model to prevent the avoidable loss of precious organs.

Preclinical animal models for chronic diseases

Many trials of biological therapies for chronic diseases, such as rheumatoid arthritis or multiple sclerosis, have been based on results from small animal models, such as collagen-induced arthritis (CIA), adjuvant arthritis (AA) or experimental autoimmune encephalomyelitis (EAE). However, in view of the substantial immunological differences between mouse and man [17], it can be debated whether the immunological mechanisms in the acute disease models represent the pathogenic mechanisms in chronic RA or MS. In retrospect, we might have to conclude from the many failures in patient trials of experimental therapies that worked well in CIA and EAE, that this is not the case. This conclusion directly implies that we need to consider which items can be addressed in the currently available models and for which aspects alternative models will have to be used or developed.

It is remarkable that, in contrast to transplantation immunology, where NHP are considered highly useful to bridge the considerable gap between rodents and patients, this is not the case in the research of autoimmune diseases. A significant advantage of non-human primates is their large size, enabling longitudinal monitoring of pathological processes by, for example, joint biopsy [24] or through imaging techniques [25]. As larger blood volumes can be collected, immune parameters in individual animals can be longitudinally monitored in the same way as for patients. By contrast, immune responses in mice are commonly measured in spleen or lymph nodes, although patterns of immune reactivity in blood and lymphoid organs can differ substantially. Besides these practical advantages, the outbred nature of NHP and the genetic, immunological, microbiological and anatomical similarity with humans substantially enhances the validity of the preclinical disease models in NHP. There are at least two areas in therapy development

where preclinical safety and efficacy tests in NHP have been highly recommended, namely for the evaluation of tolerization strategies [16] and for the development of gene therapies [26]. However, disease models in NHP are also highly useful for the safety and efficacy testing of other biological therapies, including antibodies, cytokines and cytokine antagonists.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is the most prevalent immune-mediated arthritic disease in the human population, affecting 1% of the population in a female to male ratio of 2.5 to 1. The pathological hallmarks of RA are the aggressive hyperproliferation (pannus formation) and inflammation of synovial tissue (synovitis) and the consequent progressive destruction of joint cartilage and bone. The hyperplastic synovium becomes infiltrated with blood-borne T-cells and macrophages at an early stage, significantly before warmth and swelling of the joints is observed [27,24]. The infiltrated T-cells contribute significantly to the pathophysiological cascade of reactions that gives rise to the disease and are, therefore, among the most favored targets for immunotherapy.

The fact that CD4+ T cells are clearly present in the arthritic joint and that several effective anti-arthritic drugs, such as corticosteroids or cyclosporine, act on CD4+ T cells has raised the question of whether patients would benefit from their elimination with antibodies [28]. Trials with anti-CD4 antibodies in rodent models of experimental arthritis show significant clinical benefit [29] and many groups have tested them in RA patients. Although essentially all groups have reported successful depletion of CD4 cells from peripheral blood, clinical results seem to be moderate at best. The clinical (in)effectiveness appeared to be independent of the intensity of CD4+ cell depletion from the synovium [30,31]. A broad variety of T cell-directed therapies, such as T cell vaccination, altered peptide ligands or skewing of CD4+ cell activity from a pro-inflammatory T helper 1 to an anti-inflammatory T-helper 2 type of response, has been tested in animal models and RA patients. However, although often the expected immunomodulatory effects could be observed [32,33], the effects on the disease are disappointing.

The highly successful concept of RA treatment through neutralizing the inflammatory cytokine tumour necrosis factor-α (TNF-α) was not based on extensive experimentation in animal models, but rather on the detailed understanding of the molecular pathology of the RA joint [10]. Although ~30% of the patients appear resistant to the treatment, biological inhibitors of soluble TNF-α (e.g. infliximab, etanercept and adalimumab) are now part of the current RA treatment. Recent trials also show that radiological joint scores can improve through anti-TNF-α treatment, although the most pronounced effect is on joint inflammation. The notion that TNF-α is an important pathogenic factor in RA, led to the creation of a transgenic mouse that spontaneously develops RA-like disease by the expression of human TNF-α in the joints [34]. This model has been useful for unraveling the complex cytokine cascade in the rheumatoid synovium [35], in particular the pathogenic significance of factors acting downstream of TNF-α, such as IL-1β [36]. It is important for therapy that, while being triggered by the inflammatory process, TNF-α-independent persistence of inflammation and erosive cartilage damage by IL-1β becomes increasingly important in the course of arthritis. Most of the immunotherapy research for RA by drug companies focuses now on TNF-α and IL-1β.

The disappointing results of the broad variety of T-cell directed therapies based on studies in acute arthritis models contrasts sharply with the success of the TNF-α transgenic mouse. It has been thought that if a model reproduces the clinical consequences of a chronic pathological process within the joint, this will be a decisive factor in establishing its validity. By contrast, models such as CIA and AA were primarily developed for the study of autoimmune events in the early phase of RA, for which they have been useful. However, these early events probably differ considerably from pathogenic mechanisms operating in the chronic disease.

Obvious ethical and technical reasons prohibit the creation of transgenic disease models in non-human primates, therefore we have to rely on experimentally induced models. In recent years, the rhesus monkey model of collagen-induced arthritis has proven its unique value for the safety and efficacy evaluation of a range of new anti-arthritic therapies that, for various reasons, could not be tested in lower species. These therapies ranged from small molecules to cytokines, cytokine antagonists, antibodies and gene therapy (reviewed in [26]).

Multiple sclerosis

Multiple sclerosis (MS) is a chronic progressive neurodegenerative disease of the CNS. The pathological hallmark of MS is the lesion, a focally demyelinated area with a variable degree of inflammation, axonal pathology, astrocytic scar formation and remyelination. Lesions are formed by a combined antibody- and T-cell-mediated autoimmune attack on specific constituents in CNS myelin. Whereas classical treatments aimed at suppression of the autoimmune reaction, such as corticosteroids, more-modern treatments try to skew the autoimmune reaction from a pathogenic T helper 1-driven mechanism to protective T-helper 2 responses. Recent advancements

Table 3. Effectivity comparison of immunomodulatory drugs in multiple sclerosis and experimental autoimmune encephalomyelitis

Therapeutic Intervention	EAE in rodents	Multiple sclerosis (effect on clinical disease)
Immunosuppression		
Corticosteroids	Effective	Yes
Mitoxantrone	Effective	Yes in relapsing-remissing disease (RR) and secondary progressive disease
Roquinimex	Effective	Trial aborted due to side effects
Sulfasalazide	Aggravated disease but effective in guinea pigs	No effect on expanded disability status scale
Cladribine	No reports	Yes (but conflicting results)
Adhesion molecule		
Anti-VLA-4	Yes	Yes in RR
Antigen-specific		
Oral tolerance	Efficacy dependant on model	No effect with myelin basic protein
Altered peptide ligands	Effective	Trial aborted with altered peptide ligands of the myelin basic protein
Copaxone	Effective	Effective in some patients
Cytokine		
TNF α	Effective	Worsening/ No effect
IL-10 (recombinant)	Effective in some models	Ineffective
IL-4	Gene therapy, side effects	Insufficient efficacy
TGFβ	Dependant on isoform	No effect
IGF-1	Dependant on model and regimen	No effect on MRI
Others		
IVIg	Effective in some models	Partially effective
TcR peptides	Effective in specific models	No effect
Cannabis	Effective	pain reduction
Beta interferons	Efficacy depends on model	effective in some patients
Estriol	Effective	Reduces lesions detectable by magnetic resonance imaging

in the immunopathology of MS have led to the development of a variety of biological treatments, including interferon-β, copaxone and intravenous immunoglobulin (Table 3). However, although these show moderate effects, mainly in the relapsing-remitting phase of the disease (RRMS), their effectivity in chronic MS is heavily disputed. This might be due to underlying differences between RRMS and chronic disease or that models used for drug development are not sufficiently representative of the disease spectrum in humans.

The animal model of choice for MS is EAE. A variety of mAbs and other biological drugs that were found to be effective in rodent EAE models were tested in MS, although with little success [37], reiterating that the effectivity in EAE models clearly does not predict effectivity in MS. Of greater concern is the number of unpublished trials that have failed, or that had to be halted [37]. Clinical trials are ongoing with the pan T-cell-depleting antibody CAMPATH-1H [38] and the anti-VLA-4 antibody Natalizumab [39], intended to prevent blood–brain barrier transmigration of blood-borne mononuclear cells.

MS patients are eagerly waiting for an effective treatment for their disease. We think that, as in transplantation, the selection of potential anti-MS therapies can greatly benefit from preclinical tests in currently available NHP models. We have established several unique EAE models in the common marmoset [40], which have proven their value in the preclinical testing of therapeutic antibodies against the T-cell co-stimulatory molecule CD40 [41,42] or the p40 subunit of the pro-inflammatory cytokines IL-12 and IL-23 [43]. Besides the strong clinical and neuropathological similarity of the models with chronic MS in humans, a great advantage of this model is that brain lesions can be visualized and characterized with comparable magnetic resonance imaging techniques as used in a clinical setting [25]. In our current trials we have been using these to test whether a new therapy can suppress the enlargement and activity of lesions [44], or whether engraftment of potentially myelinating cells is effective.

Conclusion

The broad variety of models for immune-based diseases established in mice and rats are of great value for the development of pathogenic and therapeutic principles and hypotheses. However, the considerable immunological distance between mice (and probably other rodent species) and humans hampers the direct translation of such principles to the human disease. We recommend that the proven validity of NHPs as an intermediate model in safety and efficacy tests in transplantation immunology will also be considered in the development of therapy for chronic diseases. That preclinical research in NHP is much more expensive than in rodents cannot be denied. However, investments made in the early detection of promising lead compounds for new drugs saves valuable time and money.

The increased demand for NHP in biomedical research raises public concerns on the justification and the consequence for the welfare of the animals [45,46]. Researchers using NHP for biomedical research should take these concerns serious and adequately inform the public – and the management of the research institutes should take all measures to ascertain that experiments in NHP can be done in an optimal setting for the animals.

Acknowledgements

We thank H. Smits and M. Vierboom for critical reading of the manuscript and Henk van Westbroek for the artwork.

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