ELSEVIER

Preclinical models of arthritic disease in non-human primates

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The costs for the development of new drugs have increased dramatically over the past 30 years. One of the main reasons for this increase is the low success rate of new drugs being approved for patient use, which is, in part, a consequence of the common use of rodent models for preclinical validation of efficacy. Especially in the development of biologicals, which are now successfully used in the treatment of rheumatoid arthritis, the selection of the right animal model is pivotal. Non-human primates could help to bridge the evolutionary gap between rodent models and human patients.

Introduction to rheumatoid arthritis

With a prevalence of 1%, rheumatoid arthritis (RA) is one of the most common immune-mediated inflammatory diseases (IMID) in the human population. Although the disease can start at any age, the peak onset is between 25 and 55 years, women being affected about three times more frequently than men. RA primarily affects the synovial joints of all extremities and less often the spinal column and is pathologically characterized by severe inflammation and progressive destruction of cartilage and subchondral bone. Recent data have shown that pathological changes occur early during the disease [1]. Synovitis can be detected before macroscopic joint-inflammation [2] and radiological evidence of joint destruction can be detected within a few years after the development of clinical signs and symptoms in most patients with active arthritis [3,4].

As with most IMID, the event(s) that initiate the disease are unknown. Nevertheless it is generally accepted that, once established, the immune system contributes significantly to the disease pathogenesis. The presence of activated T- and B-lymphocytes as well as macrophages within the early arthritic synovium supports a strong pathogenic influence of the immune system.

Biologicals in the treatment of RA

The drug development industry invests heavily in biotechnology with the objectives of identifying new targets for therapy [5] and developing safe and effective medicines on the basis of naturally-occurring regulators and antagonists of pro-inflammatory molecules [6]. The underlying idea is that owing to the high specificity, biologicals will act more specifically and have fewer side-effects than the registered broad-acting nonbiological disease-modifying and anti-inflammatory drugs. However, it has become clear that biological agents have their own safety problems that are not encountered with small molecules, such as the induction of neutralizing immune responses that prevent further action [7,8], anaphylactic shock due to an unforeseen hypersensitivity against therapeutic antibodies [9], recrudescence of latent infections [10] or the risk of a cytokine storm due to massive immune activation by the therapeutic antibody [11,12].

The availability of biologicals (antibodies and soluble receptors) that can neutralize the activity of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-1, has significantly improved the treatment of RA [13]. Both cytokines have complementary effects in animal models of RA, showing that the main effect of TNF-α is in the induction and perpetuation of joint inflammation, whereas IL-1 seems more involved in the joint destruction process [14]. In addition, antibodies directed against a variety of other inflammatory mediators, including other pro-inflammatory cytokines and chemokines, are currently being tested in clinical trials.

Although the striking success of TNF-α neutralizing agents in RA is undisputed, there are several limitations:

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1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2007.02.012

(i) In ~30% of RA patients, TNF-α blocking agents, such as infliximab (chimeric anti-TNF antibody), adalimumab (human anti-TNF antibody) and etanercept (soluble TNF receptor) have little therapeutic effect on clinical signs and symptoms.
(ii) TNF-α has a key function in the host defense against infections. Predictably, long-lasting administration of TNF-α neutralizing agents can have unwanted side-effects, such as recrudescence of latent infections (tuberculosis, hepatitis B). Moreover, complications in patients with cardiac problems have been reported [15].
(iii) Biologicals can eventually induce neutralizing antibodies that limit their efficacy in the case of chronic treatment [8].

In conclusion, although treatment of RA has been improved tremendously, there is still a need for new effective therapies for patients who do not respond or do not tolerate currently available therapeutic regimens [16].

Drug development and animal models

A common feature of most biologicals developed for the treatment of IMID is that their mode of action and high specificity precludes safety and efficacy testing in standard rodent disease models. TNF-α neutralizing agents have been directly tested in patients after promising effects had been observed in rodent models. An important step for translation of basic research into the clinic was the construction of an RA model in severe combined immunodeficient (SCID) mice, based on implanted human synovium together with human bone and articular cartilage [17,18]. The model has unequivocally demonstrated that T cells control the production of matrix-degrading enzymes and pro-inflammatory cytokines within the arthritic synovium [18,19]. The SCID model can provide important information on local mechanisms that operate in the inflamed joint and consequently identify target(s) for therapy. However, major differences remain between rodent (humanized) models and RA patients (Table 1). Moreover, there is increasing awareness that the wide immunological gap between the standard laboratory rodent models and humans contributes significantly to the high attrition rate of biologicals in clinical trials. We have discussed this issue in a previous contribution and proposed that because of the well-established physiological, anatomical, genetic, microbiological and immunological proximity with humans, monkeys provide useful preclinical models of IMID, including arthritis [20].

Of the well-established mouse and rat models of RA only the collagen-induced arthritis (CIA) model could be reproducibly induced in rhesus (*Macaca mulatta*) [21] and cynomolgus macaques (*Macaca fascicularis*) [22]. The induction of severe poly-articular arthritis normally requires a single immunization with heterologous type II collagen (C-II), which can be extracted, for example, from enzymatically digested bovine hyaline cartilage. We observed a variable sensitivity among randomly chosen animals from the large outbred colony (> 1 000 individuals) of Indian origin rhesus monkeys held at BPRC [21,23]. In ~60% of the monkeys, CIA could be induced, whereas the remaining ~40% appeared completely resistant to the disease, even when booster-immunizations were given with C-II in incomplete Freunds adjuvant (IFA). A common feature in the group of CIA-resistant monkeys was that they all shared a major histocompatibility complex (MHC) class I serotype, namely Mamu-B26 [24]. It is of note that the Mamu-B26 serotype was formally indicated as Mamu-A26, but was renamed after the observation that the serotype defines a genomic region configuration encoding *Mamu-B* molecules [25]. It is also pertinent to emphasize here that the segregation of CIA susceptibility with the Mamu-B26 serotype has been shown for Indian origin rhesus monkeys, but might not apply to colonies of other origin, for example China. The observed CIA resistance is confined to younger age groups [24], has no clear gender association and is disease-specific because Mamu-B26 positive and negative monkeys are equally susceptible to experimental autoimmune encephalomyelitis (EAE), the elected experimental model of multiple sclerosis (MS) [26]. By contrast, susceptibility of rhesus monkeys to EAE maps to the *Mamu-DP* region, which has no detectable effect in the CIA model. Although susceptibility to CIA in rodents maps to the MHC class II region, we have not found a clear influence of this genomic region on the severity or course of CIA in rhesus monkeys. This was rather unexpected because several rhesus monkey *MHC-DRB1* alleles contain the 'shared epitopes' (QKRAA, QRRAA) present in human *MHC-DRB1* alleles that are associated with RA severity [27,28].

Biomarkers of CIA susceptibility and disease course in rhesus monkeys

Biomarkers are highly useful for the monitoring of disease symptoms in laboratory animals, especially in the preclinical phase when overt clinical signs are lacking. The availability of CIA-susceptible and CIA–resistant rhesus monkeys in our colony has been instrumental for the identification of disease markers.

Genetic susceptibility markers

By preselection of monkeys lacking the Mamu-B26 serotype, a CIA prevalence of more than 95% can be achieved. The most important consequence is that experiments with a minimum group size of six animals are sufficiently powered for statistical evaluation.

Immunological susceptibility markers

A comparison of humoral and cellular immune parameters of CII-immunized Mamu-B26 positive and negative rhesus monkeys revealed several differences. The proliferative response of mononuclear cells (MNC) from peripheral blood or lymphoid organs against the immunizing CII antigen were substantially lower in Mamu-B26 positive than in Mamu-B26 negative monkeys [29]. Immunoglobulin G (IgG) antibody levels against bovine CII Mamu-B26 positive and negative monkeys were similar and the IgG reactivity with epitopes within the core CB11 fragment appeared to overlap [23,30]. However, we observed a marked production of IgM antibody against bovine CII in CIA-sensitive monkeys (Mamu-B26^{-ve}; Figure 1), while Mamu-B26+ve animals showed no production of IgM [23]. In subsequent studies we observed a close association between impaired anti-CII IgM production and disease resistance, in that case induced by pre-sensitization against attenuated bovine CII [31]. The possible underlying mechanism is that collagen in intact cartilage is largely protected from antibody binding [32]. However, binding of pentameric IgM antibody to the scarcely exposed collagen epitopes can induce classical route complement activation and neutrophil recruitment, which in concert can cause serious damage to the

TABLE 1

Nonhuman primate and rodent CIA models versus RA in humans[a]

	Rheumatoid arthritis	Rhesus monkey CIA	Rodent CIA
Epidemiology			
Nature of population	Outbred	Outbred	Inbred
Prevalence	1%	60%	100% in susceptible strains; 0–50% in nonsusceptible strains
MHC association	HLA-DR1 and –DR4	Negative association with Mamu-B26	Varies between strains
Shared epitope	Present	Present	Absent
Sex-linkage	Yes, female prevalence	No	In some models (e.g. NZB mice)
Spontaneous onset	Yes	No	In transgenic models (e.g. MLR/1 mice)
Cause(s)	Unknown; probably viral or bacterial infection	Immunization	Immunization, infection, transgene expression (TNF)
Clinical course	rarely acute, mostly relapsing-remitting or chronic progressive	Monophasic, mostly acute	Monophasic, relapsing-remitting, chronic progressive or acute
Characteristic pathology			
Macroscopic aspect	Symmetric inflammation of diarthtrodial joints	Symmetric inflammation of diarthtrodial joints	Prominent ankle swelling
Synovitis	Yes	Fulminant	Yes
Germinal center formation in synovium	Yes	Mostly diffuse infiltrates	Diffuse infiltrates in some models, focal in others [xx]
Cartilage pathology	Extensive	Extensive	Strain and/or model dependent
Bone remodeling	Present	Present	Strain and/or model dependent
Immunopathology			
Induction	Not testable	Type II collagen, (methylated) protein (AIA)	Type II collagen, bacterial antigens (mycobacteria, streptococcal cell wall), (methylated) protein (AIA)
Transferable by CD4+T cells or T cell lines	Not testable	Not tested	Yes
B cell and/or antibody involvement	Plasma cells, Ig/Complement deposits in lesions	Association CIA susceptibility with anti-CII IgM	Strain dependent, transferable with anti-CII IgG antibody
Testing of human specific immunotherapies	Possible	Possible	Only in transgenic or SCID models

[a] Abbreviations: AIA, antigen-induced arthritis; CIA, collagen-induced arthritis; CII, type II collagen; Ig, immunoglobulin; TNF, tumor necrosis factor; The validity of rodent and primates as model of IMID has been extensively reviewed elsewhere [17].

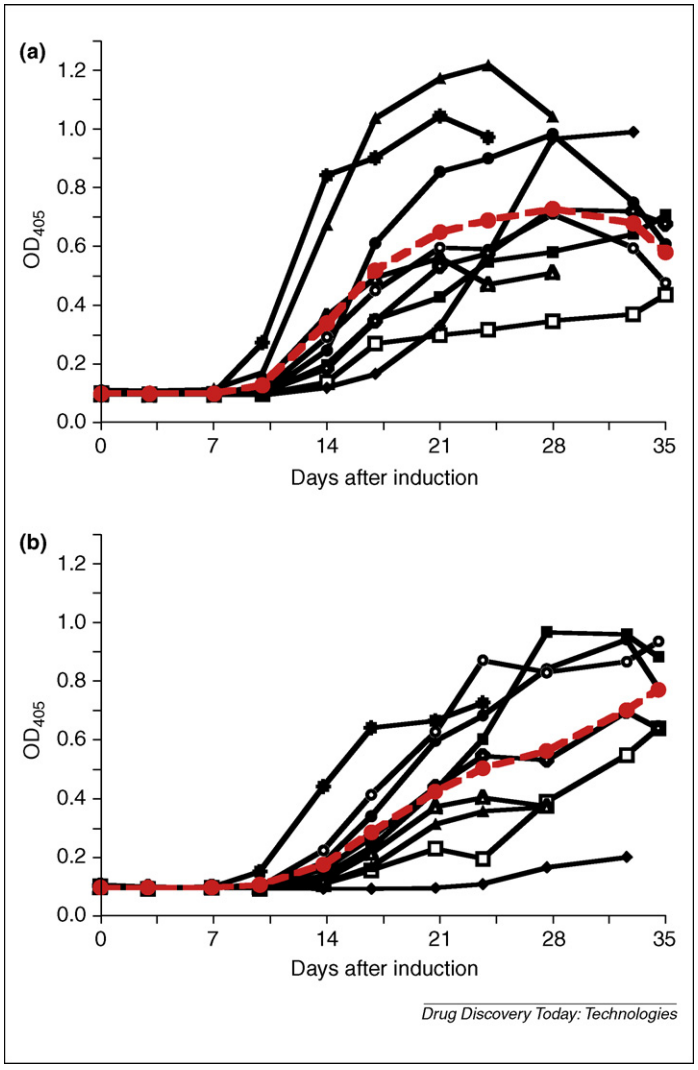


FIGURE 1

The induction of (a) IgM and (b) IgG autoantibodies directed against collagen type II (CII) in eight CIA sensitive animals (Mamu B26^{-ve}). Antibody titres were determined by ELISA. A mean trend-line for production of both antibody isotypes is given in red. Serum for the analysis of CII specific antibodies was collected twice a week. Serum for the analysis of CII specific IgG was diluted 10-fold. Serum for the analysis of CII specific IgM was diluted 100-fold. OD_{405}, optical density at 405 nm.

cartilage surface [33] and cytotoxicity to chondrocytes located in superficial cartilage layers [34]. In preclinical trials this biomarker is used to determine whether a monkey that fails to develop CIA might be resistant to the disease.

In contrast to the genetic homogeneity of rodent strains commonly used for the evaluation of the efficacy of new drugs for RA, the outbred nature of the rhesus monkeys results in a heterogeneous genetic make-up necessitating longitudinal evaluation of individual animals [26]. This is done by combining multiple disease parameters involving clinical observations and biomarkers. Biomarkers used in the evaluation of CIA have been identified, which reflect different pathological aspects of the model, including clinical markers, markers for inflammation, immunological markers and markers for cartilage degradation and bone erosion.

Clinical markers of disease

Because monkeys cannot directly inform the observer on the impact of the disease on their well being, twice weekly assessment of outward physical signs, body weight and body temperature and daily assessment of behavioral changes are performed to assess the clinical well being of the animal. These data are summarized in a value from 0 to 5 and make up an integrated discomfort scoring system (Table 2) that provides useful guidelines for the ethical management of the model. The rhesus monkey model of CIA was originally developed as a preclinical model of RA in adults and shows characteristic features of RA as defined by the American Rheumatism Association criteria (Table 3; [35]). However, the model shows also distinct similarities with juvenile forms of arthritic disease, such as the heterogenic clinical presentation [26], the monophasic course [23,36], the prominent production of IL-6 (Figure 2; [37,38]), a proinflammatory cytokine that is pivotal in the perpetuation of juvenile idiopathic arthritis (JIA) and the pathogenic role of autoimmunity against type II collagen (Figure 1; [39])

Biomarkers in the evaluation of disease activity and the effects of treatment

Once a week, a complete hematological and serological analysis is performed that provides additional information on the disease status and the general physical condition of the animal. The development of CIA and the effects of new treatments can be evaluated through the analysis of different hematological, serological and physical biomarkers.

TABLE 2

Integrated discomfort scoring table

Disease score	Characteristics	Monitoring frequency	Maximal duration
0	No disease symptoms	Daily	End of experiment
0.5	Fever (>0.5 °C)	Twice per week	12 weeks
1	Apathy, loss of appetite, weight-loss.	Daily and (for weight-loss) twice per week	10 weeks
2	Warm and tender joints, but without STS[a]	Twice per week	6 weeks
3	Moderate STS but normal flexibility of affected joints	Twice per week	4 weeks
4	Severe redness with STS of joints, with joint stiffness	Twice per week	2 weeks
5	Such severe disease that euthanasia is necessary	Daily	18 hours

[a] STS, soft tissue swelling.

TABLE 3

Compatibility of the rhesus monkey CIA model with the ARA criteria for RA [35]

Criterion[a]	Definition	Rhesus monkey CIA model
1. Morning stiffness	Morning stiffness in and around the joints, lasting at least one hour before maximal improvement.	Present[b]
2. Arthritis of three or more joint areas	At least three joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician; the 14 possible joint areas are right or left proximal interphalangeal (PIP) joints, metacarpophalangeal (MCP) joints, wrist, elbow, knee, ankle and metatarsophalangeal (MPT) joints.	Present
3. Arthritis of hand joints	At least one area swollen (as defined above) in a wrist, MCP or PIP joint.	Present
4. Symmetric arthritis	Simultaneous involvement of the same joint areas (see criterion 2 above) on both sides of the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry).	Present
5. Rheumatoid nodules	Subcutaneous nodules over bony prominences or extensor surfaces or in juxta-articular regions, observed by a physician.	Not prominent
6. Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects.	Absent
7. Radiographic changes	Radiographic changes typical of RA on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized to or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify).	Present

[a] For classification purposes, a patient is said to have RA if he or she has satisfied at least four of the seven criteria. Criteria 1 through 4 must have been present for at least six weeks. Patients with two clinical diagnoses are not excluded. Designation as classic, definite, or probable RA is not to be made.
[b] Affected joints give a prolonged stiffness.

Biomarkers of inflammation

Serum levels of C reactive protein (CRP) and the pro-inflammatory cytokine IL-6 are elevated during episodes of active disease in JIA and RA. The elevation of serum IL-6 is associated with the extent and severity of joint involvement in both diseases as well as in the CIA model [21]. Moreover, IL-6, although originally described as a terminal differentiation factor of B cells, also induces the production of acute phase proteins like CRP by hepatocytes and can be superimposed on the production of CRP (Figure 2).

CRP: CRP is an acute phase reactant that is commonly used to monitor RA disease activity and serves as an early marker for the onset of arthritic disease [40]. An increment of serum CRP levels above 50 mg/L indicates that manifest arthritis in the CIA model will follow within a few days. On the basis of the time interval between CIA induction and the onset of the CRP rise, three types of CIA responders could be discerned in an outbred group of 31 animals: early responders (n = 6) show a CRP increase before post induction day (PID) 14; intermediate responders (n = 12) show an increase between PID 14 and 21; and late responders (n = 13) show an increase between PID 21 and 35.

A sharp increase in CRP levels is associated with a marked loss of body weight as a result of inflammation-induced cachexia (also known as induced cachectic syndrome; Figure 3a). The production of the serum protein albumin is reduced during an active inflammatory response through specific downregulation by monocytic products like IL-1 [41]. Predictably, serum levels of albumin are decreased during active joint inflammation (mean maximum decrease relative to day 0; Figure 3a). The acuteness of the disease onset is associated with a higher mean maximum number of affected joints (n.o.a.j.; joints that show outward signs of inflammation) per animal in each group (Figure 3a). In the early responders the mean maximum n.o.a.j. is ~26. Intermediate responders have a mean maximum n.o.a.j. of ~16. The late responders have the lowest mean maximum n.o.a.j. of 10. Thus, time of onset is inversely correlated with the severity of disease [26].

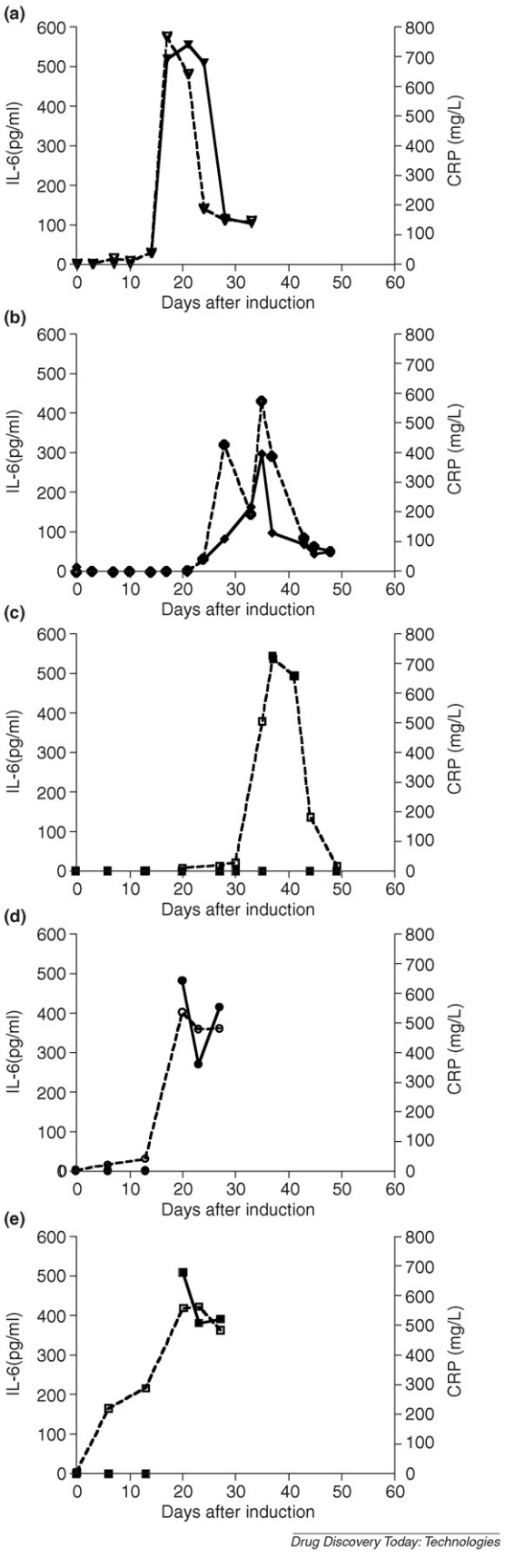
Neutrophils, platelets and hematocrit

An increase of platelets and neutrophils normally marks episodes of active inflammation. Furthermore, active periods of the disease are associated with decreased hematocrit values [26].

Biomarkers for joint erosion

The enzyme alkaline phosphatase (ALP) is produced by the liver and osteoblasts in equal proportion. Once demonstrated that liver enzymes are unaltered, ALP has been used as an indicator of osteoblastic activity where it is involved in making phosphate available for bone calcification. ALP is increased during active phases of the disease. Whether ALP isoenzymes, in particular bone-specific ALP, can be used to monitor joint destruction in the model has as yet not been assessed.

In conjunction with ALP, urinary excretion rate measurement of collagen crosslinks provides a comprehensive picture of bone metabolism and how it is affected by the disease and subsequent treatments (Figure 3b). Joint tissues contain different quantities of the major collagen crosslinks hydroxylysylpyridinoline (HP) and lysylpyridinoline (LP), which are metabolically stable degradation products of collagen contained in cartilage and bone and excreted



into the urine. About 95% of the crosslinks in the rhesus monkey joint cartilage consists of HP (HP:LP ratio = 55) while the HP:LP ratio of bone is 3.8 [36]. As the excretion rate of the crosslink product varies during the day [42,43], urine samples for analysis are collected overnight. Nonhydrolysed urine samples are used for the measurement of collagen crosslinks with reversed-phase high-performance liquid chromatography (HPLC) essentially according to Black *et al.* [44]. Increased changes of excretion rates of HP and LP, expressed relative to creatinine, are observed during the active phase of CIA (Figure 3b). In particular increased excretion rates of HP are associated with CIA severity and directly related to the number of involved joints during disease.

Preclinical intervention trials

Although the pathogenic role of T cells in arthritic diseases is undisputed, the effect of T-cell targeting therapies in patients has been largely disappointing thus far. An illustrative example of a treatment where promising effects observed in preclinical models could not be reproduced in patients with established disease is with CD4 targeting antibodies [45]. Blocking the interaction between CD28 and CD80/86, constituting a major costimulatory pathway in T cell activation, with CTLA4Ig (Abatacept) results in anergic T cells or apoptosis of T cells. In a recent clinical trial with Abatacept significant improvement was observed in patients with RA, thereby underlining a role of T cells even in ongoing disease [46]. The objective of new therapies is to hit the right target at the right time.

In the course of the past 15 years, we have evaluated a broad range of candidate therapies in the rhesus monkey CIA model, including, among others, monoclonal antibodies, cytokines, cytokine antagonists, chemokine antagonists and viral vectors for gene therapy. Not all data are in the public domain, because sometimes producers of a new treatment did not permit publication of the data. In the following we will summarize some of the (published) preclinical trials and discuss the implications for our understanding of the (immuno) pathogenic mechanism.

Interference in early disease

Using biopsies of clinically involved and non-involved joints from RA patients and CIA-affected monkeys we have shown that synovitis can precede clinically manifest swelling of the joint [2]. The early synovitis was characterized by significant infiltrates of CD3+ T-cells and CD68+ macrophages. To examine whether removal of active synovium had an impact on the subsequent disease progression, CIA-affected monkeys received intra-articular injections with an adenoviral vector that encoded thymidine kinase (TK) or lacZ as a control gene as well as for detection of the vector. The TK-encoding vector appeared to make the synovial tissue sensitive to apoptosis induction by intravenous gancyclovir and abolition of the joint inflammation [47].

FIGURE 2

IL-6 and CRP production in Mamu-B26 negative rhesus monkeys developing CIA. The IL-6 production (straight line) in five representative animals (a–e) developing CIA follows the same kinetics as the production of CRP (broken line).

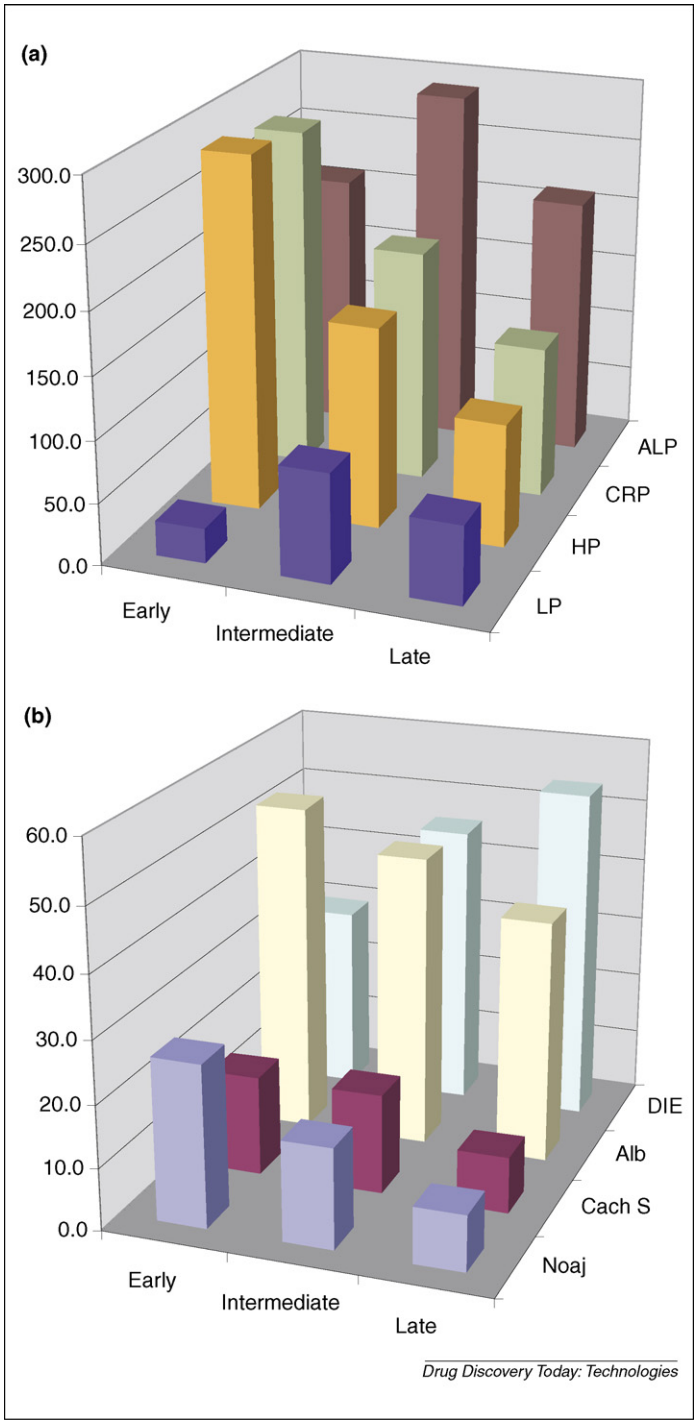


FIGURE 3

Biomarkers of disease activity in the rhesus monkey CIA model. Animals were divided into three groups on the basis of response to induction of the disease. Acute or 'early' responders show an increase of CRP before post-induction day (PID) 14 (n = 6); Moderate or 'intermediate' responders show an increase of CRP between PID 14 and 21 (n = 12). 'Late' responders show an increase of CRP between PID 21 and 35 (n = 13). **(a)** Biomarkers of joint erosion: LP, lysylpyridinoline; HP, hydroxylysylpyridinoline; CRP, C-reactive protein (mean maximum production mg/L); ALP, alkaline phosphatase (mean change in production per measured event relative to PID 0). **(b)** Biomarkers of inflammation: Noaj, mean maximum number of affected joints; Cach S, cachectic syndrome (mean maximum loss of bodyweight % relative to PID 0); Alb, albumin [mean maximum decrease (%) relative to PID 0]; DIE, days in experiment after induction.

To assess the activity window of autoreactive T-cells, we treated CIA-affected rhesus monkeys with cyclosporine A (CsA), a T-cell specific calcineurin inhibitor. The data showed that CsA treatment started before the onset of clinical signs prevented the development of CIA, whereas treatment during the active phase of the disease had no detectable effect [48]. Similar data were obtained with daclizumab, a humanized antibody directed against the Tac antigen on the IL-2 receptor α chain [49]. These data seem to contrast with the observation that, in a limited number of monkeys, the spontaneous remission of arthritis can be reverted by a booster-immunization with bovine CII in incomplete adjuvant. Interestingly, T cells from these monkeys displayed a (residual) proliferative response *ex vivo*, against CII [23].

A key pathogenic role of T cells in the early phase of CIA was further supported by the strong effect after prophylactic treatment with a small molecular CCR5 antagonist [50]. The antagonist acts by preventing the binding of CCR5 ligands and, hence, the recruitment of arthritis promoting T cells to the joint. Treated animals showed an improved control of inflammatory parameters like CRP, bodyweight and albumin, and stabilized production of alkaline phosphatase, HP and LP as biomarkers of joint destruction [50].

Interference in late disease

Treatment of CIA in four rhesus monkeys with recombinant human IFN-β (10×10^6 units/kg daily for one week; Rebif®) during the active phase of the disease showed a clear beneficial effect on clinically manifest arthritis in two monkeys and abolished arthritis in one monkey [51]. However, a clinical trial in RA patients with fibroblast-derived IFN-β (doses ranging from $0{,}6.10^6$ (2.2 μg) – 12.10^6 (44 μg) units/kg, three times weekly for 24 weeks; Frone®) combined with methotrexate failed to reproduce the promising effects of IFN-β observed in the monkeys [52]. It has to be noted, however, that the dose (μg/kg/day) given in rhesus monkeys was 100-fold higher.

Unfortunately, the biological benchmark for the treatment of RA, i.e. anti-TNF-α antibody, could not be tested in the rhesus monkey CIA model, because of a lack of crossreactivity.

In conclusion, the rhesus monkey model of CIA is a useful preclinical model for the assessment of safety and efficacy of new therapeutic compounds, bridging the gap between rodent models and human patients.

Future directions

Although CIA in rhesus monkeys is a robust preclinical model for the evaluation of safety and efficacy of novel therapies, certain experiments require a less severe and a more chronic model. To accommodate these requirements alternative arthritis models are being developed.

CIA in the common marmoset

The common marmoset (*Callithrix jacchus*) is a small-sized nonendangered neotropical primate that has gained popularity as a laboratory primate species for the modeling of human diseases [53,54]. It has been well established that rhesus monkeys immunized with myelin extracted from human central nervous system or with (recombinant) myelin proteins develop acute neurological disease whereas common marmosets immunized with the same

immunizing agents develop a chronic progressive neurological disease that resembles multiple sclerosis in clinical and neuropathological presentation [55]. We are currently testing whether, analogous to the situation in EAE, the common marmoset can also provide a model for chronic progressive CIA that more closely resembles the human situation in RA.

Antigen-induced arthritis (AIA)

For certain applications, it is not necessary to induce systemic arthritis. As an example, it is not known whether the introduction of a heterologous collagen matrix into a cartilage lesion in an inflamed joint will remain intact, whether autoimmunity against matrix components is evoked and whether the implant exacerbates arthritis. For experiments addressing such questions it is important that the onset of arthritis can be well timed and that joint inflammation remains moderately severe so that both beneficial and detrimental effects can be detected. To achieve this we implement the well-established mouse AIA model [56–59] in the rhesus monkey.

The model allows discrimination between immune responses developing against the immunizing antigens and autoimmune responses against pivotal self-antigens like collagen. In brief, monkeys are sensitized to foreign antigens, such as ovalbumin (OVA) or tetanus toxoid (TT), by inoculation with an emulsion of OVA/TT in a Th1-skewing adjuvant, being Dimethyl Dioctadecyl Ammonium (DDA) mixed with Trehalose 6,6′- DiBehenate (TDB). The reproducible induction of local inflammatory responses in the knee joint requires the generation of both humoral and cellular responses. Both parameters can be monitored during *in vivo* development of immune responses against sensitizing antigens. Serum samples, collected twice a week, can be tested for the development of OVA- and TT-specific antibodies every two weeks in an enzyme-linked immunosorbent assay (ELISA). Cellular responses against both antigens can be evaluated *in vivo* using delayed type hypersensitivity (DTH) assays two weeks after every immunization by intracutaneous injection of small amounts of the sensitizing antigens. Animals that develop significant antibody levels against the immunizing agent and have a positive DTH are injected with both antigens into the knee joint resulting in the development of local swelling of the joint.

These analyses in active inflammation can be preceded by first analyzing the biological effect of a therapeutic compound on DTH responses in presensitized animals. If treatment with this compound inhibits the development of a DTH response these animals could then be used, after a wash out period, for efficacy testing on locally induced inflammation of a joint.

Concluding remarks

CIA in rhesus monkeys is a very useful preclinical model of human arthritis. Together with the development of a CIA model in the smaller common marmoset and the milder AIA (DTH) model in the rhesus monkey we aim to fill a niche in preclinical testing of new human specific therapeutics that could reduce the attrition rates in the pharma industry. Furthermore, we aim to develop a better understanding into the processes that govern the chronicity of the arthritic process.

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