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REVIEW

Developing the next generation of monoclonal antibodies for the treatment of rheumatoid arthritis

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Rheumatoid arthritis is one of the commonest autoimmune diseases affecting 0.8% of the population. Over the last decade the treatment of this chronic disease has been revolutionized by the use of monoclonal antibodies and fusion proteins, targeting molecules like tumour necrosis factor alpha. Nevertheless, approximately one-third of subjects fail to respond to these therapies and therefore significant unmet medical need remains. Following a decade of use, clinical, government and regulatory agency expectations have changed for new antibodies therapies entering this highly competitive area. In this review, we discuss the current advances being made in antibody engineering and how they are being considered and used in the development of the next generation of antibodies to meet future expectations of healthcare providers, physicians and patients. Moreover, we discuss how pattern recognition receptors may provide new antibody tractable targets that may break the cycle of autoimmunity in rheumatoid arthritis.

Abbreviations

ACPA, anti-citrullinated peptide antibodies; ADCC, antibody-dependent cellular cytotoxicity; AE, adverse event; CDR, complementarity determining region; C_H, constant region heavy chain; C_{H1}, constant region heavy chain domain 1; C_{H2}, constant region heavy chain domain 2; C_{H3}, constant region heavy chain domain 3; CIA, collagen induced arthritis; C_L, constant region light chain; DAS, disease activity score; Fab, fragment antigen binding region; Fc, fragment crystallizable region; FcRn, neonatal Fc receptor; FDA, Food and Drug Administration; HLA, human leukocyte antigen; Ig, immunoglobulin; IL, interleukin; LPS, lipopolysaccharide (endotoxin); NLR, NOD-like receptors; PAMP, pathogen-associated molecular patterns; PEG, polyethylene glycol; PK, pharmacokinetics; PRR, pattern recognition receptor; RA, rheumatoid arthritis; s.c., subcutaneous; scFv, single chain variable fragment; SCW, streptococcal cell wall; TACI, transmembrane activator and calcium modulating and cyclophilin ligand interactor; TH17, T Helper 17 cells; TLR, toll-like receptor; TNF, tumour necrosis factor; V_H, variable heavy chain; Vκ, kappa light chain; V_L, variable light chain

History of monoclonal antibodies in the treatment of rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by inflammation of the synovial joints, which can result in pain, swelling and joint damage. It is one of the commonest autoimmune diseases with an incidence of 20 per 100 000 person-year and a prevalence of more than 500 per 100 000. In the UK, approximately 387 000 people have been diagnosed with RA accounting for 0.8% of the adult population (Symmons, 2002). Likewise in the USA, RA affects more than 2 million people. Whilst the exact pathogenesis of RA remains unclear, significant strides have been made over the last 20 to 30 years in our understanding and treatment of this disease. To date it is not known what the

triggers are that break immune tolerance and drive autoimmunity; however, it is known that genetic association of human leukocyte antigen HLA-DR1 and HLA-DR4 infers a 50% risk of individuals developing RA. Currently 50–80% of RA patients have the autoantibody rheumatoid factor, typically IgM and IgA molecules that bind Fc fragments of IgG, and/or anti-citrullinated peptide antibodies (ACPA). Research has shown that presence of ACPA is a good predictor of rapid disease as defined by progressive joint destruction (van der Helm-van Mil *et al.*, 2005). Moreover, Huizinga *et al.* (2005) have demonstrated a link between ACPA-positive patients and a number HLADRB1 alleles and a common shared epitope within these HLA alleles. This has led to the hypothesis that citrullinated peptides, following post-translational modification of amino acids on certain proteins, possibly due to viral infections or local injury in healthy individuals, bind to these shared epitopes on HLA alleles, resulting in the breaking of tolerance and generation of autoantibodies (Hill *et al.*, 2003). Obviously, this hypothesis does not fit within all RA, for example ACPA negative patients; however, it does begin to provide us with a model of how tolerance may be broken in the majority of subjects. Once established, however, a range of inflammatory, macrophages, neutrophils, T/B cells and stromal cells, such as synovial fibroblasts, appear to be central to the mechanisms of joint inflammation and disease progression. At the sites of joint inflammation a broad panel of mediators, such as tumour necrosis factor alpha (TNFα), interleukin (IL)-6, granulocyte macrophage colony-stimulating factor, matrix metalloproteinases, vascularendothelial growth factor, receptor activator of nuclear kappa-B ligand cc chemokine ligand 2 and cc chemokine ligand 5 (reviewed in Feldmann *et al.*, 1996), are all elevated contributing to increased inflammation, angiogenesis (due to the hypoxic nature of the inflammation) and joint damage thus highlighting the extremely complex nature of the disease. This persistent inflammation also results in systemic disease, with elevated acute phase proteins and increased circulating levels of cytokines such as IL-6 which contributes to the overall manifestation and morbidity of this condition.

Due to the chronic and debilitating nature of the disease this condition is associated with a lower quality of life, disability, premature death and unemployment. Consequently, the human, economic and healthcare costs are considerable. For example the direct real life costs for treating patients with RA in France range from €6450 to €19618 per person, with the overall cost to the French National Health Authority of €222 million (Maravic, 2010). Approximately 84% of these costs are attributable to biologic therapies such as anti-TNFs. Moreover, within the UK the indirect and direct costs of RA are estimated at between £3.8 and £4.75 billion per year (Pugner *et al.*, 2000). It has also been shown that these indirect costs increase with disease severity, from approximately €5000 for minimal disease activity up to €20 000 in patients with severe disease (Lajas *et al.*, 2003; Kobelt *et al.*, 2008). To put these figures in context the total economic cost to society in Europe and the USA has been estimated to be €45.3 and €41.6 billion respectively (Lundkvist *et al.*, 2008).

Prior to the use of monoclonal antibodies and fusion proteins in RA, a large number of small molecules ranging from broad spectrum anti-inflammatories, such as non-steroidal anti-inflammatories and analgesics to anti-malarials, anti-metabolites, alkylating agents and especially glucocorticoids were used in various combinations to manage pain and inflammation in this disease in an attempt to slow down disease progression. Whilst these drugs provided a degree of efficacy they were often associated with significant side effects adding to the burden of co-morbidities often affecting these patients. As such, a clear need for targeted therapies aimed at the modification of the disease process with an acceptable safety profile in patients with RA was sought. Like the treatment of most chronic and disabling diseases this is a not a new concept, with the idea of a 'magic bullet' being proposed by Paul Ehrlich over 100 years ago. Ehrlich originally postulated the existence of specific receptors that bind to certain antigens and hypothesized that these receptors either associated with cells or distributed in the blood in response to an antigen interaction (reviewed in Drews, 2004). From this idea he evolved the concept of specific drugs that go straight to their intended target, hence the 'magic bullet'. One of the key breakthroughs in reducing this concept to practice came much later through the pioneering work of Kohler and Milstein (1975) in which they demonstrated for the first time that B cell precursors could be immortalized through fusion with mouse myeloma cells to generate stable monoclonal antibody producing cells. The ability of administered therapeutic antisera to treat bacterial infections had been established in 1891 by Emil von Behring and Shibasaburo Kitasato, but it wasn't until this identification of a method for isolating monoclonal antibodies of a defined specificity that the potential of this approach as a drug option became a real possibility. Early clinical studies in cancer paved the way with humanized antibodies like CAMPATH-1H (alemtuzumab) (Hale *et al.*, 1988) demonstrating that by targeting an overexpressed specific antigen, such as CD52, one could provide clinical benefit.

By the mid 1980s early 1990s basic research in RA had begun to identify TNFα (Buchan *et al.*, 1988; Brennan *et al.*, 1989; Feldmann *et al.*, 1990; Deleuran *et al.*, 1992; Thorbecke *et al.*, 1992; Williams *et al.*, 1992) as potentially one of the key targets contributing to disease in RA. *In vitro* studies using human primary synovial membrane cultures from RA joints identified that these cells spontaneously produced TNFα and had high levels of IL-1 (Buchan *et al.*, 1988; Brennan *et al.*, 1989; Deleuran *et al.*, 1992). Furthermore, it was shown that IL-1 production was TNFα-dependent by blocking IL-1 release using an anti-TNFα antibody in synovial membrane cultures (Brennan *et al.*, 1989). Since IL-1 was thought to be of significant importance in inflammatory arthritis, blocking its production using neutralizing anti-TNFα antibodies could be of clinical benefit. *In vivo* evidence which strengthened the hypothesis came from studies which investigated the effect of TNFα in a mouse model of RA, collagen induced arthritis (CIA). Repeated administration of recombinant TNFα increased severity and incidence of disease relative to control mice (Thorbecke *et al.*, 1992). Conversely intraperitoneal administration of a hamster anti-mouse TNFα monoclonal antibody (15 $mg \cdot kg^{-1}$) reduced paw swelling and clinical score when administered prophylactically and also therapeutically (Thorbecke *et al.*, 1992; Williams *et al.*, 1992). This effect was found to be dose-dependent as administration of the antibody at 2.5 $mg \cdot kg^{-1}$ had little effect on paw swelling or proportion of joints with severe lesions (Williams *et al.*, 1992). In summary, these studies had shown that TNF blockade reduces IL-1

production *in vitro* in human primary synovial cultures and in mouse models of arthritis would reduce disease pathology.

Based on these observations Feldmann and Maini took the step to evaluate infliximab (cA2), a chimaeric anti-TNF antibody, in a small cohort of RA patients (Elliot *et al.*, 1993). Following single infusions of infliximab at 20 mg·kg^{-1} they were able to show in this small cohort of subjects, a significant reduction in signs and symptoms of the disease. Larger double-blinded placebo-controlled clinical trials confirmed that infliximab could produce response rates in patients with RA superior to methotrexate alone and could have a significant effect on disease progression (Maini *et al.*, 1999). Consequently, infliximab was the first monoclonal antibody to be approved for the treatment of RA patients with an inadequate response to methotrexate while maintaining a generally acceptable safety profile. This breakthrough in the treatment of RA with targeted biologic therapies to TNF has become one of the largest biologics success stories, with a market now estimated at greater than $8 billion per annum globally and five anti-TNF molecules (infliximab, etanercept, adalimumab, golimumab and certolizumab) approved either in the USA or globally. Moreover, the confidence gained from using anti-TNF therapies as a first line biologic has allowed rheumatologists to evaluate and successfully gain approval for a number of other antibody or fusion protein-based therapies such as rituximab (B cell depletion therapy), abatacept (inhibition of co-stimulation) and tocilizumab (anti-IL-6 receptor therapy). In conjunction with standard of care with small molecules, such as methotrexate and glucocorticoids, this has given rheumatologists a greater degree of clinical options to improve treatment of their patients and attain levels of efficacy not previously achieved. This is exemplified by the fact that more than 1 million patients have been treated with anti-TNF therapies. In trials irrespective of method of TNF inhibition, the combination of methotrexate with anti-TNF routinely achieve significant efficacy with 62% and 69% of patients achieving a 50% improvement in their disease at 1 year (Klareskog *et al.*, 2004; Breedveld *et al.*, 2006). Furthermore, radiographic progression was also effectively stopped in the majority of RA patients treated in combination. For example in the TEMPO study (Klareskog *et al.*, 2004) the mean change in radiographic progression, as defined by total sharp score, at week 52 was –0.5 (–1.5 to 0.0, 95% confidence interval) in the combination-treated group when compared with 2.8 (1.08 to 4.51, 95% confidence interval) in the methotrexate alone group.

Whilst anti-TNF and additional biologics therapies have clearly revolutionized the treatment of RA, and arguably the use of biologics in the pharmacopeia, there is still a significant need for new therapies that provide additional benefit, since nearly one-third of patients receiving anti-TNF therapy fail to respond to the initial treatment. Furthermore, many patients discontinue TNF because of adverse events (AE) or the development of a secondary resistance and a gradual loss of effectiveness to these agents (Finckh *et al.*, 2006) due in part to immunogenicity. One of the most important AEs associated with the use of TNF therapy is the increased risk of infection. Screening, by chest X-ray, skin testing or whole blood assays, is standard practice to reduce the chance of reactivation of latent mycobacterium tuberculosis, a known risk (Dixon *et al.*, 2010). Moreover, meta-analysis from clinical studies and RA registries clearly demonstrates that patients on TNF therapy are at a higher risk for bacterial, fungal or viral infections (Hyrich *et al.*, 2008; Leombruno *et al.*, 2009; Strangfeld *et al.*, 2009). In addition to this the debate also continues that inhibition of TNF may also lead to an increased chance of malignancy (Symmons and Silman, 2004); however, the data to date is equivocal. Currently all approved biologics in RA (except abatacept which has an infection risk warning in the label), carry black box warnings highlighting serious infections and in some cases malignancies as key risks factors. As these new therapies are recombinant proteins one of the key factors in drug effectiveness is dependent on whether the patient generates an immune response and human anti-human antibodies to the protein. For example, it is reported that approximately 10% of patients treated with infliximab were positive for autoantibodies to infliximab. Furthermore, antibody-positive patients were more likely to have higher rates of drug clearance, reduced efficacy and experience infusion reactions.

Therefore, considerable opportunity still remains to identify new, safer, less immunogenic and improved therapies to treat RA. In spite of this unmet medical need in RA there is also a clear recognition by the pharmaceutical industry that the expectations of governmental agencies (www.nice.org.uk, CG79 Rheumatoid Arthritis: full guideline), regulatory authorities, pharmaceutical competition, clinicians and patients, as a consequence of a decade of successful use of anti-TNF therapy, and more recently B cell depletion, inhibition of co-stimulation and IL-6 receptor pathway discussed later in this article, has considerably changed market expectations for new antibody therapies in this disease. Therefore, these various healthcare bodies are seeking new therapies with greater efficacy, improved safety characteristics, reduced costs and improved dosing intervals. In this review we outline some of the novel approaches being considered to meet future expectations for the treatment of RA and describe how these pressures have also paralleled an evolution in how monoclonal antibodies are engineered and developed.

Antibody structure

The basic unit of all antibodies is the immunoglobulin (Ig) molecule, a disulphide bond-linked tetramer, comprising two identical large chains of approximately 50 kDa in size, called heavy chains, and two identical smaller chains of approximately 25 kDa in size, called light chains (Figure 1). Each heavy chain is made up of four protein domains, a variable (V_H) domain which is directly involved in antigen binding and thus is specific for a given antibody, along with three constant (C_H) domains which are common to a specific antibody class and are involved in antibody functions such as cytotoxicity (MacLennan *et al.*, 1970), complement fixation (reviewed in Raghavan and Bjorkman, 1996) and serum maintenance (reviewed in Ghetie and Ward, 2000). Similarly, each light chain is made up of two domains, a variable (V_L) domain which together with the V_H domain comprises the antigen binding pocket of an antibody and a single constant domain (C_L) which is also common to all antibodies of a given class and which is bound to the N-terminal C_H domain (called C_{H1}) via a disulphide bond formed between cysteines

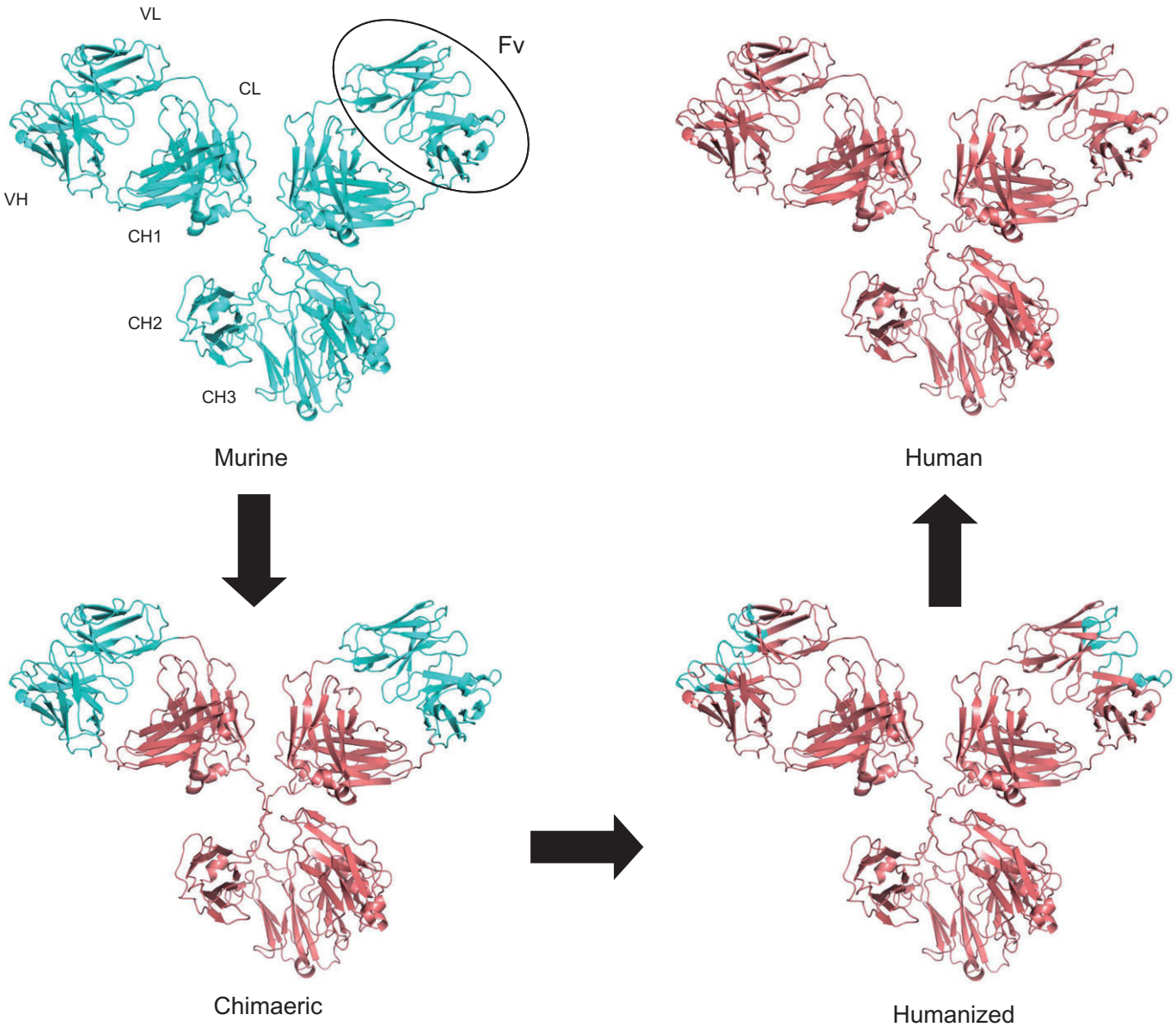

Figure 1

Evolution of therapeutic antibody development. The original monoclonal antibodies such as Orthoclone OKT3 were murine proteins. Later developments include chimaeric antibodies such as infliximab, which is made up of a murine Fab genetically fused to a human Fc region as well as humanized antibodies, whereby murine complementarity determining regions (CDRs) are grafted onto human IgG frameworks. More recently human antibodies, such as adalimumab have been developed using phage display or transgenic methods.

in these two domains. This symmetrical modular structure of Ig molecules has been central to the key developments in antibody engineering that have revolutionized the field over the last 25 years, and that has been the driving force for the recent expansion in the development and commercialization of antibody therapeutics.

Engineering monoclonal antibodies

Following Kohler and Milstein's invention of the hybridoma method for immortalizing immunized B lymphocytes, it was quickly shown that the new monoclonal antibodies could provide a degree of clinical benefit (Hale *et al.*, 1988). Initial studies proved challenging due to the onset of human anti-mouse neutralizing antibodies due to the recognition of the non-self murine protein. If one could overcome this immunogenic response it was hypothesized that the use of monoclonal antibodies might have broader utility in autoimmunity (Herzog *et al.*, 1987; Horneff *et al.*, 1991a,b; Wendling *et al.*, 1993).

By using the newly emerging field of molecular biology to re-engineer mouse antibodies with functionally equivalent human amino acids, initially by replacing the murine

Ig constant domains with human equivalents (Figure 1), the overall immunogenicity of the molecule could be reduced (Knight *et al.*, 1995). This led to a generation of so-called 'chimaeric antibodies' (Boulianne *et al.*, 1984; Morrison *et al.*, 1984; Neuberger *et al.*, 1985) with a number of these types of antibodies, such as infliximab and rituximab gaining successful approval in RA in 1999 and 2006 respectively. Nevertheless, immunogenicity due to human anti-chimaeric antibodies, hypersensitivity and consequently infusion-associated reactions, as described in their black box warning label, and in some cases a resultant lack of efficacy remained common issues with these antibodies. A key breakthrough to surmount these effects was the development of complementarity determining region (CDR) loop grafting by Winter and colleagues (Jones *et al.*, 1986). This led to the generation of new so-called 'humanized' monoclonal antibodies with minimal murine amino acids (Figure 1) in their sequences (Jones *et al.*, 1986; Verhoeyen and Riechmann, 1988). Currently the only humanized antibody approved for the treatment of RA is tocilizumab, an anti-IL-6R antibody (Nishimoto and Kishimoto, 2008), although a number of these formats have been approved in oncology (trastuzumab, bevacizumab), asthma (omalizumab) and multiple sclerosis (natalizumab).

The generation of recombinant antibodies of fully human origin was realized with the development of two alternative technologies; phage display of human antibody fragments (McCafferty *et al.*, 1990), and transgenic mice expressing human Ig genes (Green *et al.*, 1994; Lonberg *et al.*, 1994).

Phage display of antibody fragments

Using filamentous bacteriophage displaying an antibody fragment comprising the V_H and V_L domains linked together with a flexible amino acid linker sequence (known as a single chain variable fragment or scFv) as a fusion with one of its coat proteins, McCafferty *et al.* (1990) demonstrated that specific phage could be recovered on the basis of antigen binding *in vitro*. Using this approach large libraries of human V_H and V_L gene sequences were prepared from peripheral blood lymphocytes from naive non-immunized donors. Using such libraries, large panels of scFv could be rapidly enriched for antigen binding and then screened for functional activity, in an analogous fashion to small molecule chemical libraries. Using this approach Cambridge Antibody Technology (now MedImmune Ltd) and Abbott Laboratories developed the first completely human monoclonal antibody, adalimumab, to gain approval by the Food and Drug Administration (FDA) in 2003 for the treatment of patients with moderate to severe RA.

Transgenic mice

In parallel with this development other groups employed the use of transgenic mice capable of producing fully human Igs, this concept first being hypothesized as early as the mid 1980s (Alt *et al.*, 1985). Over the next decade a range of transgenic mice were developed containing mixtures of mouse and human V_H and V_L genes (Taylor *et al.*, 1992; 1994), ultimately culminating in the generation of mice with four germ line modifications, two targeted disruptions of endogenous mouse heavy and κ light chain genes and the introduction of human heavy and κ light chain transgenes (Green *et al.*, 1994; Lonberg *et al.*, 1994). Therefore, by combining early work by Kohler and Milstein (1975) with this latest discovery one would be able to generate fully human V_H Vκ IgGs as drug molecules in the mouse. As with phage display, these discoveries provided the basis for commercialization, leading to the formation of the companies Abgenix and Medarex. To date four new fully human monoclonal antibodies derived from this technology have been approved by the FDA, panitumab (Abgenix/Amgen), for the treatment of metastatic colorectal cancer (Jakobovits *et al.*, 2007), golimumab (Medarex/Centocor) as a new subcutaneous anti-TNF therapy approved for the treatment of RA (Smolen *et al.*, 2009), psoriatic arthritis and ankylosing spondylitis, ofatumumab an additional fully human CD20 antibody for the treatment of chronic lymphocytic leukaemia (Lemery *et al.*, 2010) and ustekinumab for plaque psoriasis (Tan *et al.*, 2010). Both phage display and transgenic mice are now well validated and robust technologies for the generation of high potency human antibodies and consequently most of the growing number of antibodies entering clinical trials are now completely human in origin (Reichert *et al.*, 2005).

Antibody fragments as therapies

One key limitation of monoclonal antibody therapies (infliximab, adalimumab, rituximab and golimumab) for RA is their costly manufacture compared with small molecule-based pharmaceuticals, due partly to their production via mammalian cell fermentation. There is therefore much activity towards moving to cheaper production methods, such as bacterial expression of antibodies. The most advanced product based on these new antibody formats is certolizumab pegol (Figure 2), which has been approved for the treatment for RA and Crohn's disease in the USA (certolizumab has not been approved for the treatment of Crohn's disease by the European Medicines Agency). By utilizing only the Fab' fragment of a humanized antibody to TNFα, this molecule can be expressed in *Escherichia coli* obviating the need for mammalian cell-based expression. Expression of full Ig molecules is more difficult in bacterial systems, due to the oxidizing environment of the bacterial periplasm, which is not readily conducive to the formation of the multiple cysteine bonds that order the tertiary structure of the molecule. Additionally, post-translational modifications, notably glycosylation, are not readily achieved in bacterial host systems.

Certolizumab, being a recombinant Fab' fragment, is claimed to exhibit high expression by *E. coli* fermentation. The lack of an Fc region (Ig C_{H2} and C_{H3} domains), which maintains serum antibody levels via interaction with the neonatal Fc receptor (FcRn) (Roopenian and Akilesh, 2007), results in Fab fragments having serum half-lives of less than 24 h (Weir *et al.*, 2002). Therefore, in order to maintain an economical and practical dosing regime, certolizumab needed to be modified to extend the serum half-life. To that end a 40 kDa polyethylene glycol (PEG) moiety was coupled

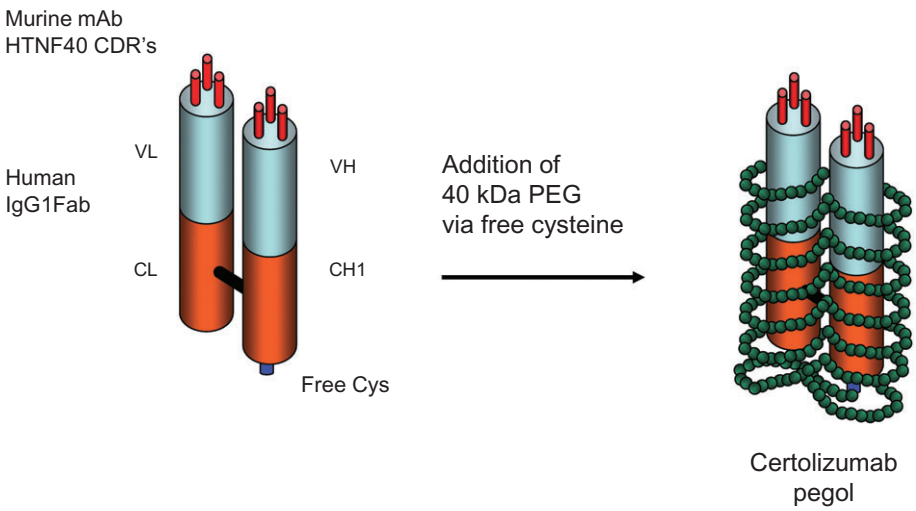

Figure 2
Molecular structure of certolizumab pegol. CDR, complementarity determining region; PEG, polyethylene glycol.

to the Fab' fragment via a free (unbonded) cysteine left from the hinge region of the original Ig sequence, resulting in a molecule with significantly extended serum persistence (Weir *et al.*, 2002).

Therefore, over the last decade of treatment of RA we have already seen how commercial competition, innovation and clinical requirements have applied evolutionary pressure on research and development strategies for monoclonal antibody therapies for the treatment of RA. As a consequence of this, we have a broad range of biologics and antibodies approved for the treatment of RA (Table 1).

Targeting the next generation of therapies in rheumatoid arthritis

Whilst significant unmet medical need remains within RA, there is also a realization by governments, healthcare providers, regulators and patients that new therapies must not only tackle the signs and symptoms of the disease, but they must also be easily delivered and cost-effective. Moreover, they must clearly demonstrate additional benefit over existing therapy to justify their use. As a consequence of their plasticity, antibodies make ideal molecules to 'tailor' these drugs to very specific requirements, that is, protein therapeutics by design.

As discussed, we have already witnessed the second generation of anti-TNF therapy, such as golimumab (Centocor), a subcutaneously delivered anti-TNF to overcome the short comings of the companies original chimaeric iv formulated product (infliximab; Centocor). The ability of companies to drive new antibody therapies with improved bioavailability has been realized by a robust understanding of human IgG pharmacokinetics (PK) (Ternant and Paintaud, 2005) and our ability to increase the strength of the antibody antigen interaction through mutation of the variable regions and selection for higher affinity variants, in a process akin to affinity maturation *in vivo*.

Affinity maturation

A fundamental principal of *in vitro* protein engineering platforms, such as phage, yeast or ribosome display is the ability to generate large numbers of variants containing mutations in controllable positions in the protein sequence, coupled with the ability to select for those variants with a defined characteristic, such as affinity for a given target (reviewed in Carter, 2006; Dufner *et al.*, 2006). A wide range of mutagenesis strategies are available, ranging from random approaches, such as error-prone PCR (Hawkins *et al.*, 1992) or DNA shuffling (Stemmer, 1994) to more targeted approaches, whereby one or more of the CDR loops is mutated (recent examples including Steidl *et al.*, 2008; Gerhardt *et al.*, 2009). Variants with improved binding kinetics can be isolated by tailoring the selection conditions such that they are preferentially enriched, for example by lowering the concentration of the antigen (Hawkins *et al.*, 1992; Schier *et al.*, 1996), or increasing the time of incubation of with antigen (e.g. Boder *et al.*, 2000). Using such approaches has led to many examples of *in vitro* matured antibodies with affinities beyond those naturally found in the immune response, with several examples exhibiting equilibrium dissociation constants in the femtomolar range (Boder *et al.*, 2000; Steidl *et al.*, 2008).

Antibody pharmacokinetics

As noted above, *in vitro* affinity maturation strategies have resulted in antibodies with extremely strong affinities for their given antigen, but to understand whether this increase will provide clinical benefit, biotech and pharmaceutical companies increasingly use theoretical models of antibody PK (Agoram, 2009). The strength that can be attributed to these models is largely based on the predictable nature of antibody PK. The half-life of human IgG is typically between 14 and 21 days primarily due to the antibodies pH-dependent

Table 1

Currently approved antibody and antibody based therapies for the treatment of subjects with rheumatoid arthritis

Generic name	Target antigen	Antibody format	Route of administration/frequency	Company	Date of approval for rheumatoid arthritis
Infliximab	TNFα	Chimaeric IgG1	i.v. infusion/3 mg·kg^{-1} every 8 weeks	Centocor Ortho Biotech Inc.	1 Apr 1999[†]
Etanercept*	TNFα	Soluble fusion protein of p75 extra cellular domain + IgG1 Fc domain	s.c. dose/50 mg weekly	Amgen/Pfizer (formerly Immunex/Wyeth)	2 Nov 1998
Adalimumab	TNFα	Human IgG1	s.c. dose/40 mg eow	Abbott Laboratories	31 Dec 2002
Golimumab	TNFα	Human IgG1	s.c. dose/50 mg monthly	Centocor Ortho Biotech Inc.	29 Apr 2009
Certolizumab pegol	TNFα	Pegylated Human Fab'2	s.c. dose/400 mg weeks 0, 2 and 4 followed by 200 mg eow	UCB Inc.	18 Nov 2009[‡]
Rituximab	CD20	Chimaeric IgG1	i.v. infusion/2 × 1000 mg 2 weeks apart. Subsequent courses every 24 weeks or based on clinical evaluation	Genentech Inc./Roche/Biogen Idec	28 Feb 2006[§]
Tocilizumab	IL-6R	Humanized IgG1	i.v. infusion/4 mg·kg^{-1} monthly or up to 8 mg·kg^{-1} monthly based on clinical response	Genentech Inc./Roche	8 Jan 2010
Abatacept*	CD80 & CD86	Soluble fusion protein of CTLA-4 + IgG1 Fc domain	i.v. infusion/500 mg (<60 kg), 750 mg (60 to 100 kg), 1000 mg (>100 kg) dosed at weeks 0, 2 and 4 and then monthly thereafter	Bristol Myers Squibb/Biogen Idec	23 Dec 2005

*Both molecules are technically not monoclonal antibodies; however, as they include the Fc domain of IgG1 and have been approved in the treatment of rheumatoid arthritis these molecules have been included.

[†]Infliximab was first approved by the FDA on 28 August 1998 for the treatment of moderate to severely active Crohn's disease and the treatment of fistulizing Crohn's disease.

[‡]Certolizumab pegol was first approved by the FDA on 22 April 2008 for reducing the signs and symptoms of Crohn's disease and maintaining clinical response in adult patients with moderately to severely active disease.

[§]Rituximab was first approved by the FDA on 26 November 1997 for the treatment of patients with relapsed or refractory low-grade or follicular, B cell non-Hodgkin lymphoma.

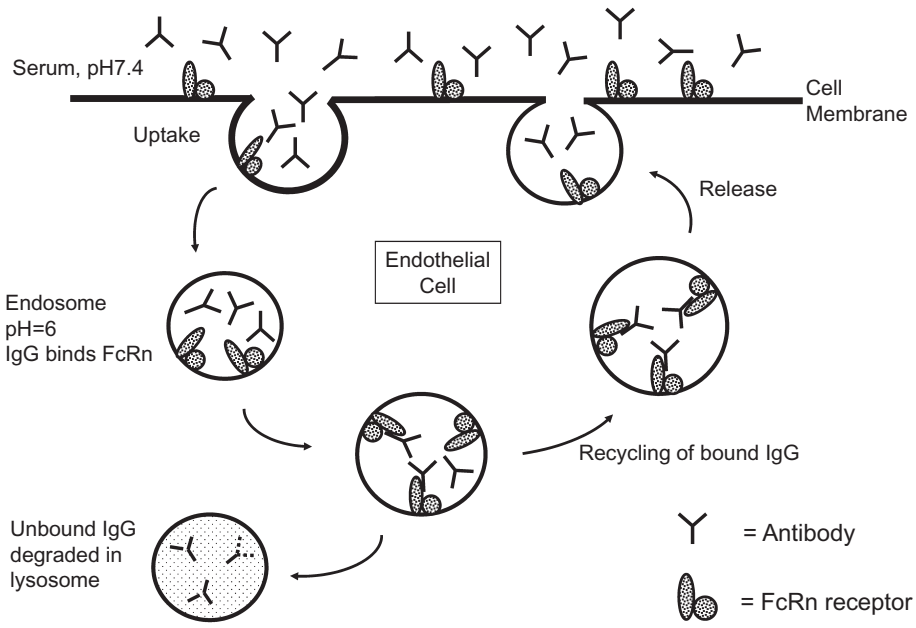

Figure 3
Theoretical model of neonatal Fc receptor (FcRn)-based IgG recycling.

interaction with the FcRn and its molecular weight (~150 kDa) preventing rapid clearance through the renal filtration. Free IgG, that is, not bound to antigen, in the circulation is passively taken up by endothelial cells through endocytosis and compartmentalized within cells in endosomes. As the pH drops from pH 7.4 to pH 6 within the endosome the free IgGs bind in a pH-dependent manner to FcRn on the endosomal membrane protecting those antibodies from degradation through lytic vesicles. As these endosomes are recycled back to the cell's plasma membrane the pH then is restored to neutral, its affinity for FcRn reduces and the antibody is released back into circulation (Figure 3).

Within RA the utility of infrequent dosing for subcutaneous formulations has been adopted by clinicians and patients to reduce the number of injections with s.c. dosing gradually moving from weekly dosing (etanercept), to once every 2 weeks (adalimumab) and most recently, with golimumab, to monthly dosing. Therefore, there is clear evidence of a need for reduced dosing intervals. One way this is being considered is through pioneering work by Dall'Acqua *et al*. (2006). They hypothesized that by increasing an antibody's binding affinity to FcRn then theoretically the serum half-life should be extended. By mutating the Fc domain of human IgG1 they identified three residues M252Y/S254T/T256E that increased the pH-dependent affinity of Fc 10-fold from 2249 ± 53 nM to 210 ± 56 nM for FcRn and consequently extended the antibody's half-life ($T^1/_2$) three- to fourfold in cynomolgus monkeys from 5.7 ± 1.4 days to 21.2 ± 9.1 days in non-human primates. Likewise, more recently additional residues have been identified (Yeung *et al*., 2009; Zalevsky *et al*., 2010) that can also extend half-life. If these *in vivo* studies allometrically scale to man, then it is possible that we could soon see anti-cytokine therapies with once quarterly dosing intervals. As yet no data has been reported in humans although clinical studies are underway (clinical trial.gov, NCT00578682) in healthy volunteers for an anti-respiratory syncytial virus antibody with extended half-life. Nevertheless, preclinical studies have been reported with an anti-IL-6 antibody subcutaneously delivered antibody with extended half-life (Moisan *et al*., 2009) as a potential therapy for the treatment of inflammation, autoimmune diseases and cancer.

Bispecifics

The key strength of monoclonal antibodies is their high specificity for their target molecule. However, for complex diseases like RA it is perhaps unlikely that any one single cytokine or molecule is responsible for driving the pathology. Therefore, to potentially increase the efficacy of new therapies in RA with antibodies, one may need to consider targeting multiple mechanisms. Due to the high cost of manufacture of these agents, dosing two independent antibodies is not currently a viable option. To overcome this hurdle, many groups have begun to consider ways in which more than one pathway can be targeted at the same time with a single antibody by generating molecules that are bi- or multispecific (Wu *et al*., 2007; Dimasi *et al*., 2009). To date, only one bispecific molecule has been approved for therapeutic use, catumaxomab, a bispecific chimaeric rat/mouse antibody targeting EpCAM and CD3, which has been approved for treatment of malignant ascites in Europe (Fresenius Biotech/TRION Pharma). However, approaches such as this are showing extremely promising preclinical findings in many different disease indications.

Whilst targeting multiple pathways seems like an obvious next step, identifying the most appropriate partnership is challenging as it assumes that both the pharmacodynamics

and PK are identical for each arm of the antibody, something that is not necessarily the case. Moreover, early attempts at using two biologics anti-inflammatories in RA, for example anakinra + etanercept, resulted in a significant increase in incidental infections that was greater than standard of care (Genovese *et al.*, 2004). Thus targeting multiple pathways may also increase side effects and in some way begin to resemble the side effect profiles typically associated with the development of new small molecule compounds.

Increased effector function

Whilst anti-TNF therapies have revolutionized the treatment of RA, one criticism of these agents is that they do not directly address the key question of autoimmunity and only target a downstream effector of inflammation, rather than the core cause of the disease. Professor J. Edwards hypothesized that to tackle autoimmunity one must first address the source of the autoantibodies which stimulate the perpetuation of an inflammatory response, and therefore target those self-perpetuating B cells responsible for the production of these autoantibodies (Edwards and Cambridge, 1998). Using rituximab (a B cell depleting therapy then licensed for the treatment of non-Hodgkin lymphoma) in patients with RA, this group demonstrated profound and lasting responses for at least 6 months after a single treatment cycle of two 1 g infusions in five patients. This result was later confirmed in a randomized Phase IIa study involving 161 patients with active RA where rituximab provided significant clinical benefit and that this response was associated with a significant decrease in autoantibodies such as rheumatoid factor and anti-cyclic citrullinated peptide (Edwards and Cambridge, 2001; Leandro *et al.*, 2002; Edwards *et al.*, 2004). The most interesting observation was that the response was sustained for 7–33 months after a single treatment cycle and that the failure to respond or disease relapse was often associated with insufficient depletion of autoantibody producing B cells or return of circulating B cells, respectively, thus clearly establishing a link between the B cell and disease pathology (Cambridge *et al.*, 2006; Cohen *et al.*, 2006; Edwards *et al.*, 2006; Leandro *et al.*, 2006; Popa *et al.*, 2007). Consequently, the chimaeric antibody rituximab was approved for the treatment of RA in 2006. This approach demonstrated that by identifying a specific target antigen one could remove a specific subset of cells and provide clinical benefit. Thus a further two CD20 B cell depleting antibodies are currently in clinical development (ofatumumab and TRU-015).

With the success of rituximab a number of approaches are being considered to enhance effector function to either improve efficacy and/or increase the duration of pharmacodynamic activity, resulting in infrequent dosing regimens. Engineering the Fc domain of monoclonal antibodies has resulted in a number of antibody variants with enhanced ADCC (antibody-dependent cellular cytotoxicity) activity when compared to wild-type antibodies. This is typically achieved by engineering amino acids in the Fc domain (Lazar *et al.*, 2006) or by modifying the carbohydrates at the glycosylation site Asn297 in the Fc domain (Shinkawa *et al.*, 2003). Thus a number of second generation CD20 B cell depleting antibodies are currently in clinical development for both non-Hodgkin lymphoma and RA. However, as rituximab depletes greater than 90% of peripheral blood B cells in RA, it is not clear how improvements in ADCC will ultimately lead to significant improvements in the clinical response. Alternative approaches to improve efficacy in RA by B cell depletion have targeted different B cell antigens such as CD19 (CLB-CD19, MEDI-551) and CD22 (*Epratuzumab*) which are more broadly expressed in the B cell lineage. Recently, an afucosylated CD19 antibody (MEDI-551) with enhanced effector function has started a Phase I clinical trial for the treatment of systemic sclerosis (clinicaltrials.gov, NCT00946699), and therefore it will be interesting to determine whether targeting CD19 demonstrates any significant differences in efficacy or safety over therapies such as rituximab.

Autoimmunity and pattern recognition

As discussed previously, targeting TNF was and still remains, a major breakthrough in the treatment of RA. However, over time it has become apparent that many individuals do not respond to these therapies or respond in such a minor way to provide only a modicum of clinically measurable benefit that does not translate into a meaningful improvement in their condition. Consequently, new therapies continue to be trialled in RA. New potential approaches currently in development include targeting the macrophages and granulocyte macrophage colony-stimulating factor pathway (MedImmune Ltd, Nycomed, Morphosys), IL-17A pathway (Ely Lilly, Novartis, Amgen) and additional anti-B cell therapies, anti-B lymphocyte-stimulating factor (HGS/GSK), anti-CD22 (UCB), TACI-Ig (Zymogenetics/Serono) and anti-CD20 approaches (GSK/Genmab/trubion). Many of these therapies are designed to specifically address the signs and symptoms of the disease, but it has also been shown that treating patients early enough in their disease progression can result in attainable clinical remission within RA (Quinn *et al.*, 2005). Therefore, over the next decade a greater emphasis is likely to be placed on therapies that also have the potential to induce remission (Smolen *et al.*, 2010).

The theory that an unresolved bacterial or viral infection provides the initial trigger for RA has been hypothesized for many years (Gibson, 1928; Cecil and Nicholls, 1931). As our understanding of the immune response has advanced this hypothesis may now be revised in the context of pattern recognition receptors (PRRs). PRRs such as toll-like receptors (TLRs) and NOD-like receptors (NLR) are expressed on a variety of cells of both myeloid and non-myeloid lineage. For the purposes of this review we will concentrate on TLRs as NLRs are intracellularly expressed and are therefore not yet an antibody tractable target class. Since their identification in 1997 (Medzhitov *et al.*, 1997), TLRs have become integral to our understanding of innate (Janeway and Medzhitov, 2002) and to some extent adaptive immune responses (Iwasaki and Medzhitov, 2004; Reynolds *et al.*, 2010). This family of PRRs (of which there are now 10 human members) recognize various microbial pathogen-associated molecular patterns (PAMPs) (Medzhitov and Janeway, 1997, O'Neill *et al.*, 2009). However, the scope of TLRs are not restricted to detecting microbes such as bacteria, fungi and viruses as numerous

endogenous 'ligands' have been proposed (Bauer *et al.*, 2009; McCormack *et al.*, 2009; Drexler and Foxwell, 2010). Therefore, in the context of PRRs the infection theory can be revised thus: microbial ligands acting through PRRs induce the release of inflammatory mediators, for example TNFα/IL-1β which cause cellular and tissue damage. This resultant damage induces the release or up-regulation of inflammatory proteins some of which contain damage-associated molecular patterns or 'Danger signals' (Matzinger, 1994) which may then drive the autoimmune pathogenesis of RA by acting back through PRRs (van der Heijden *et al.*, 2000; McCormack *et al.*, 2009). Therefore, targeting TLRs with neutralizing antibodies may break this inflammatory loop and could be a therapeutic approach in the treatment RA.

Expression of TLR2 and 4 have been confirmed in RA synovium (Radstake *et al.*, 2004; Ospelt *et al.*, 2008), RA peripheral blood monocytes (Iwahashi *et al.*, 2004) and cells from RA synovial fluid (Seibl *et al.*, 2003; Huang *et al.*, 2007). Furthermore, *in vitro* stimulation of RA synovial cells demonstrates that these cells are hyper-responsive to both TLR2 and TLR4 microbial ligands [peptidoglycan and lipopolysaccharide (LPS) respectively], when compared to normal cells (Huang *et al.*, 2007). Further evidence for the involvement of TLRs in RA is the inhibition of spontaneous TNF production from human synovial membrane cultures by overexpression of dominant negative versions of TLR2/4 adaptor molecule MyD88 and Mal (Sacre *et al.*, 2007). Moreover, endogenous TLR4 ligand came from studies investigating IL-1β and TNF-induced joint inflammation (Abdollahi-Roodsaz *et al.*, 2009). Washouts from murine patellas stimulated with IL-1β induced murine TLR4 complex-dependent IL8 production from transfected human embryonic kidney cells. Furthermore, this response was inhibited by the TLR4 antagonist *Bartonella quintana*.

Numerous *in vivo* studies have strengthened the hypothesis of the role of pattern recognition in arthritis. Zymozan (a known TLR2 ligand) induced arthritis was significantly reduced in TLR2–/– mice (Frasnelli *et al.*, 2005). In a streptococcal cell wall (SCW) induced arthritis model TLR2–/– mice did not develop joint swelling or inhibition of cartilage synthesis whereas TLR4–/– mice developed joint inflammation similar to wild-type mice (Joosten *et al.*, 2003). Subsequent studies which looked at both early and late time points in SCW-induced arthritis reproduced this data for the acute phase (days 1–2) of the disease, with a clear protective effect in TLR2–/– mice (Abdollahi-Roodsaz *et al.*, 2008a). However, the chronic phase (day 28) joint inflammation was significantly reduced in TLR4–/– and not TLR2–/– mice. The authors suggested that the shift in dependence from TLR2 to TLR4 may correlate with the shift from innate to adaptive immune response. This type of studies may provide an insight into the initial events that trigger RA; however, it would be virtually impossible to treat this initial event from a pharmaceutical perspective because it is unknown which pathogens might trigger this event or identify which proportion of subjects that would in turn be predisposed to develop RA. However, evidence that endogenous ligands signalling through PRRs maybe be involved in established disease can be gained from models using adenoviral expression of IL-1 and TNF locally in joints to induce joint inflammation and damage. In this system it was shown that IL-1-driven inflammation was significantly reduced in TLR4–/– and, but to a lesser extent, TLR2–/– mice. In contrast, TNF-induced inflammation was reduced to a lesser extent with TLR4–/– mice (Abdollahi-Roodsaz *et al.*, 2009). Therefore, it was not blockade of the initial pro-inflammatory stimulus (i.e. IL-1) that reduced inflammation but blockade of endogenously released TLR ligands in response to IL-1. This is supported by the observation that TLR4–/– mice are protected in the IL-1Ra–/– spontaneous arthritis model (Abdollahi-Roodsaz *et al.*, 2008b). Interestingly, TLR2–/– mice in this model appeared to exacerbate in this system clearly highlighting that work is needed to fully understand the role of these pathways in RA. In addition, it was also hypothesized that pattern recognition may also be involved in driving T Helper 17 (TH17) cell and IL-17 release in this system, which is a known clinical target in RA currently under clinical investigation. This hypothesis has in part been recently substantiated in that a TH17 mouse autoimmune experimental autoimmune encephalomyelitis model has been shown to be TLR2-dependent (Reynolds *et al.*, 2010). Antibody intervention studies in models of arthritis have not as yet been reported; however, the effects of a TLR4 antagonist (*B. quintana*) has been evaluated in both the CIA model and the IL-1Ra–/– arthritis model (Abdollahi-Roodsaz *et al.*, 2007). It is believed that LPS from this pathogen acts as a TLR4 antagonist by competing with other TLR4 agonists and preventing signalling through the TLR4/MD2 complex, although this has as yet not been formally demonstrated. In these models *B. quintana* attentuated arthritis in both models whether dosed prophylactically or therapeutically and reduced IL-1β levels in the CIA arthritic joint. This type of approach is currently being evaluated in the clinic with a PhIII study in severe sepsis (Clinicaltrials.gov NCT00334828) (Kim *et al.*, 2007; Park *et al.*, 2009; Tidswell *et al.*, 2010) but as yet has not been trialled in autoimmune disease.

These above data suggest the possible involvement of the role of pattern recognition in driving the pathogenesis of RA. It is of note that microbial ligands have been detected in RA synovial joints (van der Heijden *et al.*, 2000) although they have also been detected in non-arthritic joints (Schumacher *et al.*, 1999). Whatever the trigger, be it microbial PAMPs or inflammatory cytokines, the search is on to identify an endogenous 'ligand' which drives RA. Numerous 'proposed' TLR ligands have also been detected and there is some compelling evidence for their involvement in the inflammation observed in RA. A by no means exhaustive list would include heat shock proteins, fibronectin, hyaluronan, high-mobility group protein-B1 (reviewed in Miyake, 2007; O'Neill, 2008; Bauer *et al.*, 2009; Huang and Pope, 2009; Andersson and Harris, 2010) and tenascin C (Midwood *et al.*, 2009; Erridge, 2010; Goh *et al.*, 2010). The issue with all of these studies is the ability to convincingly show that the observed effect of the ligand is not due to LPS contamination (Tsan and Gao, 2007).

Despite the strong rationale for TLRs in RA, there have been no studies investigating the inhibitory effects of antibodies in this disease or *in vivo* models of RA. Neutralizing anti-mouse TLR2 (Meng *et al.*, 2004) and TLR4 (Akashi *et al.*, 2000) are commercially available. Moreover, potent anti-mouse (Daubeuf *et al.*, 2007) and anti-human TLR4 MAbs (Dunn-Siegrist *et al.*, 2007) developed by NovImmune are currently in pre-clinical studies and it would be interesting to speculate how these would perform in the various models of

induced and spontaneous arthritis. Targeting a receptor involved in bacterial sensing would of course also have safety implications in terms of host defence and these would need to be monitored accordingly. Interestingly, a small clinical RA study involving 23 subjects with active disease [disease activity score (DAS) 28 > 3.2 at baseline] treated with chaperonin 10 (XToll™), a downstream inhibitor of TLR signalling, suggested a significant improvement in disease activity, as determined by DAS28, from baseline to day 84 (Vanags *et al.*, 2006) irrespective of dose. However, as this was not a placebo-controlled study, further studies are warranted to determine the relative significance of this change in DAS. Nevertheless, these support the notion that inhibition of TLR signalling may provide clinical benefit in these subjects.

Whether antibody inhibitors of TLRs will drive subjects into remission is another question. Pattern recognition is the first event involved in mounting an inflammatory response to a pathogen; moreover, once established, the resulting cellular damage and endogenous ligands may continue to signal through these self same receptors perpetuating the inflammation. In RA it has been hypothesized that this process is involved in helping to break tolerance and initiating autoimmunity. It has already been demonstrated that a window of opportunity exists to treat early RA to increase the proportion of patients achieving remission (Quinn *et al.*, 2005). Whilst this effect has been demonstrated, this approach cannot be used in clinical practice as rheumatologists are only able to prescribe biologics until a DAS28 > 5.1 plus failure of two synthetic disease modifying antirheumatic drugs has been demonstrated. Nevertheless, with this key observation the recognition that timing is crucial may see a step change in how biologics are used earlier in the treatment paradigm, and therefore antibody therapies directly targeting pattern recognition might be able to break the early cycle of disease and help promote remission in these subjects. Perhaps as our understanding of the endogenous mediators driving RA and the role that PRRs play in this develops, biologics can once more revolutionize the treatment of RA perhaps addressing the aetiological basis of disease rather than signs and symptoms of current therapies.

Conclusion

Whilst the treatment of RA has improved significantly over the last two decades, considerable unmet medical need remains not just to treat signs and symptoms but also to generate new therapies that may promote disease remission. In concert with changes in clinical practice in RA, the use of monoclonal antibodies has already evolved to keep pace with the requirements of the physician and patient. The exploitation of the inherent 'plasticity' of antibodies coupled with recent improvements in molecular engineering has led to the ability to dial in and out particular properties depending on the mechanism of action and target that the therapy is being designed for. Therefore, over the next decade a new generation of antibody therapies will be developed for the treatment of RA that will hopefully provide increased efficacy and remission rates, improved safety characteristics, as well as be better tailored to the requirements of patients, physicians and healthcare providers.

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Conflict of interest

JC, DL and MS are all employees of MedImmune Ltd (a wholly owned subsidiary of AstraZeneca). MedImmune currently has a number of monoclonal antibodies in preclinical and clinical development for the treatment of RA (CAM-3001), systemic lupus erythematosus (MEDI-545) and systemic sclerosis (MEDI-551).

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