Dealing with immunogenicity of biologicals: assessment and clinical relevance

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Purpose of review

In the last decade, biologicals revolutionized rheumatology. An increasing number of patients benefit from biotherapeuticals. However, some patients do not respond to treatment and others lose their response after a certain time. Immunogenicity is one of the factors linked to secondary nonresponse but its clinical significance has remained controversial.

Recent findings

In recent years, knowledge of how to assess immunogenicity of biologicals has improved. Various reports show an inverse relationship between drug levels and antibody formation against the drug. Studies associated immunogenicity of therapeutic antibodies with clinically significant nonresponse in a subgroup of patients. Clinically relevant immunogenicity is influenced by several factors including dosing and concomitant medication. It has been shown that immunogenicity against biologicals can be persistent or transient.

Summary

Immunogenicity affects a significant number of patients treated with biologicals. Monitoring of drug levels as well as of antibodies against therapeutic antibodies may lead to more rational treatment strategies.

Keywords

biologicals, immunogenicity, personalized medicine, therapeutic drug monitoring

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Introduction

In the last decade biologicals have revolutionized clinical rheumatology. The term biological is used for therapeuticals produced by biotechnology. Biologicals can be native proteins like hormones, cytokines, and growth factors or engineered molecules such as therapeutic antibodies, antibody fragments or proteins constructs. Today, monoclonal antibodies are the fastest growing class of human pharmaceuticals. More than thirty antibodies and antibody-derivatives have been approved worldwide. Several hundreds more are being investigated in clinical trials in various therapeutic indications including oncology and autoimmune disease.

In rheumatology the therapeutic antibodies infliximab and adalimumab and the receptor construct etanercept are widely used as treatment for rheumatoid arthritis, ankylosing spondylitis, and psoriatic arthritis [1–3]. Moreover, new biologicals such as rituximab, an anti-CD20 antibody and abatacept, an anti-CD80/anti-CD86 fusion protein, have become available [4,5]. Many more bio-therapeuticals are in late stage of development for clinical use.

Immunogenicity refers to antibody formation against a certain drug. All biologicals can induce an unwanted immune response [6].

The immune response against native biologicals differs from the immune response against designed biologicals containing new foreign epitopes. An immune response against native human hormones, growth factors and cytokines occurs only when the natural tolerance against these biologicals is broken. The frequency of this type of immunogenicity is low; it may, however, have serious consequences such as severe anemia in the case of antibody formation against erythropoietin [7].

Immunogenicity against designed biologicals reflects more the normal immune response against a foreign intruder. The first antibodies used as therapeutical were of mouse origin. Some 90% of patients treated with murine antibodies produced human antimouse antibodies (HAMA) [8]. This hampered their clinical use. Immunogenicity of therapeutic antibodies has been reduced by replacing murine constant regions with human ones, resulting in chimeric antibodies such as infliximab and rituximab. These antibodies have been

shown to induce the formation of human antichimeric antibodies (HACA). Humanization of the variable regions further reduced immunogenicity. However, even fully human antibodies may lead to the production of human antihuman antibodies (HAHA) [8,9]. Antibody formation against human therapeutic antibodies is usually directed against the idiotype of the molecule.

Immunogenicity of fusion proteins like etanercept and lenercept depends on their similarity to native proteins. The fusion part of these biologicals may contain new epitopes that can be recognized as foreign by the immune system. In the case of lenercept clinically relevant antibody formation against the fusion part of the molecule has been described [10]. Also for etanercept, the label indicates antibody formation to etanercept. However, two recent studies did not detect any clinical significant antibody formation to etanercept [11,12], indicating that immunogenicity is not an important issue for etanercept.

Assessing immunogenicity

One of the major obstacles in assessing the clinical relevance of immunogenicity is the complexity of measuring antibodies against antibodies. An antibody response is diverse, ranging from low-affinity IgM antibodies to high-affinity IgG1 antibodies. None of the currently available assays is able to detect all different forms of antibodies. Moreover, all assays differ in specificity and sensitivity. Many straightforward enzyme-linked immunosorbent assay (ELISA) techniques suffer from nonspecific binding. In recent years two improved types of assays for determining immunogenicity have been used to detect immunogenicity: the two-site (bridging) assay and the antigen binding test, a radioimmunoassay (RIA) [13].

The two-site assay uses the monovalency of the two arms of IgG1, 2 and 3 to crosslink a labeled biological to a biological coated on an ELISA plate. This assay is specific and sensitive, but does not detect IgG4 antibodies, since IgG4 is bispecific molecule due to exchange of half molecules [14].

In the RIA system, IgG from patient serum is immobilized to a solid phase. Consequently, a radiolabeled drug is captured by drug-specific IgG (if present) from the fluid phase. This type of assay has a low background and is able to detect clinically relevant antibodies [15].

It is clear that differences in assays hamper the comparability of studies on immunogenicity. Standardization and comparison of the different assays in relation to clinically relevant immunogenicity therefore is an important issue.

The second problem encountered in assessing immunogenicity is inhibition of the assay by the presence of the drug in the serum. Many biologicals are used chronically. Administration of the drug to patients with antibody formation will result in immune complex formation. The half-life of IgG antibodies is approximately 3 weeks; the half-life of immune complexes, depending on their size, is much shorter [16]. Immune complex formation thus will accelerate the clearance of the applied drug and the antidrug antibody. The detection of antidrug antibodies therefore depends on the relative amounts of antidrug antibodies produced and the amount of the administered drug (see Fig. 1). Free antidrug antibodies are not detected if an excess of drug is present in the serum. A minor antidrug response will only result in lowering of the drug level. When equal amounts of drug and antibody are present, neither drug levels nor antibody levels will be detectable. If the production of antibodies exceeds the amount of the drug in the serum, all drug applied is cleared from the circulation and only free antibody to the drug can be measured.

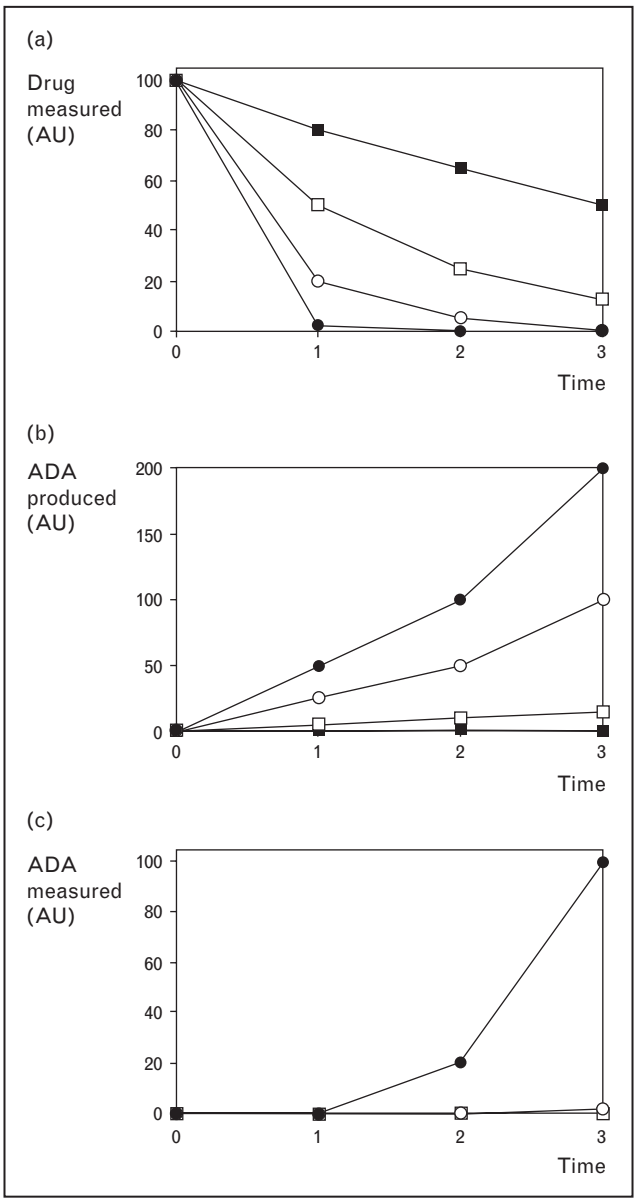
As a consequence of this, the interpretation of antibody measurements should be linked to the timing of administration and dosing of the drug. A rational strategy for immunogenicity assessment is to start with determining the drug level in the serum. If this appears to be unexpectedly low, testing for antidrug antibodies should be performed. This also indicates that the frequency of antibody responses in the patients usually is underestimated.

The third complicating factor in assessing immunogenicity is the circumstance that immunogenicity is a gradual process, developing and changing over time. Continuation of treatment may either induce tolerance or stimulate further antibody production. Antibodies against infliximab or adalimumab have been described to become undetectable in some patients upon continuation of treatment or dose escalation [17,18•]. In a large study with long follow-up on multilple sclerosis patients treated with the therapeutic human antibody natalizumab, antibodies against this compound were detected in 9% of the patients, in 3% these antibodies were transiently positive and in 6% persistently positive. Persistently positive patients showed a loss of clinical efficacy compared with antibody-negative patients. In transiently positive patients, full efficacy was achieved after approximately 6 months of treatment, the time when patients were becoming antibody-negative [19•].

Allergic reactions

Patients who develop antibodies to biologicals are more likely to show infusion-related reactions. Acute infusion reactions, including anaphylaxis, develop in a close

Figure 1 Model for relationship between drug antibodies administered, antidrug-antibodies (ADA) produced and antidrug antibodies measured



The graphs show (a) drug levels measured, (b) antidrug antibodies produced and (c) antidrug antibodies measured. Four different situations, depending on the relationship between drug administered and antibodies produced. First, no antidrug antibodies (closed square, no ADA). Second, the amount of antibodies produced is not enough to clear all drugs after 3 weeks but trough levels are reduced (open square, ADA $<$ D). Third, the amount of ADA is enough to clear all drug at 3 weeks (open circle, ADA $=$ D). Fourth, all drugs are rapidly cleared by antibody formation, no drug level and high titer of ADA at week 3 (closed circle, ADA $>$ D).

temporal relationship to an infusion. The acute reactions can be truly allergic, namely IgE-mediated type I reactions, including hypotension, bronchospasm, laryngeal or pharyngeal edema, wheezing and/or urticaria. In patients with Crohn's disease an increased risk of infusion reactions was observed in patients with higher antiinfliximab levels [20]. In a small study using radio labeled infliximab it was demonstrated that in addition to the quantity of antiinfliximab, the quality of the response is related to infusion reactions [21]. Many of the antiinfliximab antibodies are of the IgG4 and IgG1 isotype. IgG4 antibodies are considered to be less inflammatory as they do not activate the complement system. In a study of 19 patients with infusion reactions to infliximab indeed an association with the level of antiinfliximab antibodies was observed. However, no protective effect of specific IgG4 was found [22]. In patients who receive biologicals subcutaneously local injection reactions are frequently seen; the relation to antibody formation is, however, unclear. In some patients treated subcutaneously with biologicals a systemic response is observed [23], but little information is available on the clinical effects of chronic immune-complex formation in these patients. In patients receiving rituximab, immunogenicity has been linked to a delayed type of hypersensivity reaction with purpura that mimics a vasculitis-like syndrome [24].

Clinical response and pharmacokinetics

The major clinical consequence of the development of antibodies is altered pharmacokinetics. The normal half-life of IgG1 is around 3 weeks. Immune complexes are cleared faster from the circulation [16,21]. As a result an inverse relationship between drug levels and antidrug antibodies is created.

A single gift of the chimeric infliximab induces antibody formation in the majority of patients. In patients treated with infliximab for Crohn's disease antiinfliximab antibodies were detected in 61% of the patients [20]. This antibody formation was associated with reduced serum trough levels, allergic reactions and a reduced response to treatment. The relevance of immunogenicity is considerably diminished by continued treatment. Maini *et al.* [25,26] demonstrated that antiinfliximab formation is lower in rheumatoid arthritis (RA) patients receiving higher dosages of infliximab. Patients treated with 10 mg/kg infliximab had significantly less antibody formation compared with patients treated with 3 or 1 mg/kg (7, 21, 53% respectively). A further reduction of antiinfliximab formation was seen in patients receiving low-dose methotrexate. In the ATTRACT study 2 years of follow-up did not reveal clinically significant antiinfliximab formation in RA patients [26]. More recently, observational studies in RA patients receiving 3 mg/kg infliximab did show a relationship between immunogenicity and reduced response to treatment [17,27,28]. These differences in part might be explained by the fact that most of the patients in the ATTRACT study used higher dosages of infliximab. In ankylosing spondylitis also higher dosages of infliximab are applied (5 mg/kg every

6 weeks), however, without concomitant methotrexate [29]. Recently, antiinfliximab formation was associated with a loss of response in a subgroup of patients with ankylosing spondylitis [30]. These findings indicate that loss of response due to antiinfliximab formation is a common phenomenon that occurs frequently in every routine practice. Awareness of immunogenicity is limited due to the lack of availability for appropriate testing.

For the fully human antibody adalimumab earlier studies reported differently on the clinical significance of immunogenicity. The Armada trial did not find any clinically significant effect of antiadalimumab formation in RA patients treated with adalimumab [2]. Van de Putte *et al.* [31] reported that 12% of adalimumab treated patients tested positive for antibodies against adalimumab. No differences in adverse events were found. However, the ACR20 response rate was numerically lower for patients who were positive for adalimumab. In a recent study investigating the relation between clinical response pharmacokinetics and antiadalimumab formation it was shown that formation of antiadalimumab is associated with lower serum adalimumab concentrations and nonresponse [18•]. This study in an observational cohort of 121 patients reported that antiadalimumab antibodies were detected in 21 patients (17%) during 28 weeks of treatment. Thirty-four percent of patients who failed to respond to adalimumab had antiadalimumab antibodies, compared with only 5% of those who responded to adalimumab. Patients with antibodies during follow-up had lower serum adalimumab concentrations at 28 weeks compared with patients who did not have antibodies. Eighty-four percent of patients who did not develop antiadalimumab antibodies received concomitant MTX, compared with only 52% of those who developed antibodies. The Japanese CHANGE investigators observed a similar relationship in a double blind randomized controlled trial comparing three dosages of adalimumab monotherapy [32]. The percentages of patients who had at least one antiadalimumab-positive serum sample from the start of study drug treatment until 30 days after the last dose were 42, 44, and 26% in the 20, 40, and 80 mg adalimumab groups, respectively. Antiadalimumab positive patients had significantly lower adalimumab levels. At week 24, the antiadalimumab-positive patients (14, 28 and 35%) in the adalimumab 20, 40, and 80 mg groups showed lower ACR20 response rates than antiadalimumab–negative patients (39, 57, and 57%). Taken together, these data indicate that immunogenicity influences the outcome of a significant number of patients treated with adalimumab, especially when given as monotherapy.

Immunogenicity, clinical relevance

During recent years our understanding of immunogenicity of biologicals and its clinical relevance have increased significantly. It has become clear that immunogenicity should be measured with specific assays and that measurements of the pharmacokinetics should be included for interpretation of test results.

An immune response probably occurs in most patients treated with therapeutic antibodies [33]. In many patients, however, the response may not be strong enough to significantly alter pharmacokinetics. Moreover, tolerance will be obtained in some, but not in other individuals. Important factors influencing the development of antibody formation are dosage and concomitant medication.

Whether this is due to a kind of immunological tolerance or simply reflects the capacity of the immune system to produce antibodies is unknown.

Next to dosage and concomitant medication other factors are likely to influence immunogenicity as well. Genetic factors like polymorphisms of HLA and immune-regulating molecules like IL-10 have been linked to influence immunogenicity in hemophilia patients who develop antibodies to factor VIII [34,35]. At present little is known about the role of genetic factors in RA patients treated with biologicals.

Immunogenicity has implications for the clinical application of biologicals.

First of all it is important to prevent clinically relevant immunogenicity by use of concomitant immunosuppressive medication. In RA patients treated with therapeutic antibodies this is frequently done. However, in diseases like ankylosing spondylitis and psoriasis therapeutic antibodies are often given without concomitant medication. Remarkably, clinical trials in these diseases provide little detail on the issue of immunogenicity [29,36]. Studies comparing treatment strategies with and without MTX or higher dosages might be warranted.

The second consequence is the need for therapeutic drug monitoring in patients treated with therapeutic antibodies. Nowadays most biologicals are given on the basis of a 'one size fits all' strategy. Adaptation of treatment strategy is usually performed based on clinical judgment only. Considering the natural variability of most inflammatory diseases and the large interindividual variation of pharmacokinetics in patients treated with biologicals, a more personalized treatment seems to be advisable. Therapeutic drug monitoring in patients, which includes disease activity measurements as well as testing for drug and antibody levels, will most probably lead to more effective treatment.

In patients with very high drug levels a prolonged treatment interval seems to be indicated. Patients with drug

levels below the therapeutic level may benefit from increased dosages. Patients with high levels of antidrug antibodies may benefit from switching to a similar type of treatment with a different protein. Nonresponders with drug levels above the therapeutic level will probably benefit more from switching to an alternative mechanism.

Conclusion

In recent years, knowledge of how to assess immunogenicity of biologicals has improved. Several studies have shown that immunogenicity is associated with allergic reactions and loss of response in a significant number of patients treated with chimeric or human therapeutic antibodies. Monitoring of drug levels as well as of antibodies against therapeutic antibodies may lead to more rational treatment strategies.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 301).

1 Elliott MJ, Maini RN, Feldmann M, *et al.* Repeated therapy with monoclonal antibody to tumour necrosis factor alpha (cA2) in patients with rheumatoid arthritis. Lancet 1994; 344:1125–1127.

2 Weinblatt ME, Keystone EC, Furst DE, *et al.* Adalimumab, a fully human antitumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. Arthritis Rheum 2003; 48:35–45.

3 Moreland LW, Baumgartner SW, Schiff M, *et al.* Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. N Engl J Med 1997; 337:141–147.

4 Edwards JC, Szczepanski L, Szechinski, *et al.* Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med 2004; 350:2572–2581.

5 Genovese MC, Becker JCMD, Schiff M, *et al.* Abatacept for rheumatoid arthritis refractory to tumor necrosis factor inhibition. N Engl J Med 2005; 353:1114–1123.

6 Schellekens H. Immunogenicity of therapeutic proteins: clinical implications and future prospects. Clin Ther 2002; 24:1720–1740.

7 Casadevall N, Nataf J, Viron B. Pure red-cell aplasia and antierythropoietin antibodies in patients treated with recombinant erythropoietin. N Engl J Med 2002; 346:469–475.

8 Hwang WY, Foote J. Immunogenicity of engineered antibodies. Methods 2005; 36:3–10.

9 Clarck M. Antibody humanization: a case of the 'Emperor's new clothes'? Imm Today 2000; 8:397–402.

10 Christen U, Thuerkauf R, Stevens R, Lesslauer W. Lenercept immune response to a recombinant human TNFR55-IgG1 fusion protein: auto-antibodies in rheumatoid arthritis and multiple sclerosis patients have neither neutralizing nor agonist activities. Human Immunology 1999; 60:774–790.

11 de Vries MK, van der Horst-Bruinsma IE, Nurmohamed MT, *et al.* Immunogenicity does not influence treatment with etanercept in patients with ankylosing spondylitis (AS). Ann Rheum Dis. 2008 Ann Rheum Dis. 2008 [Epub ahead of print]

12 Dore RK, Mathews S, Slechtman J, *et al.* The immunogenicity, safety, and efficay of etanercept liquid administered once weekly in patients with rheumatoid arthritis. Clin Exp Rheumatol 2007; 25:40–46.

13 Aarden L, Ruuls SR, Wolbink GJ. Immunogenicity of antitumor necrosis factor antibodies: towards improved methods of antiantibody measurement. Curr Op Imm 2008; 4:431–435.

14 van der Neut Kolfschoten M, Schuurman J, *et al.* Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. Science 2007; 317:1554–1557.

15 Bendtzen K. Immunogenicity of anti-TNF antibodies. In: van de Weert M, Møller EH, editors. Immunogenicity of biopharmaceuticals. Springer; 2008:189–203.

16 Rojas JR, Taylor RP, Cunningham MR, *et al.* Formation, distribution, and elimination of infliximab and antiinfliximab immune complexes in cynomolgus monkeys. J Pharmacol Exp Ther 2005; 313:578–585.

17 Wolbink GJ, Vis M, Lems W, *et al.* Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. Arthritis Rheum 2006; 54:711–715.

18 • Bartelds GM, Wijbrandts CA, Nurmohamed MT, *et al.* Clinical response to adalimumab: relationship to antiadalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis. Ann Rheum Dis 2007; 66:921–926.
This study shows that immunogenicity is also clinical relevant for patients treated with human antibodies.

19 • Calabresi PA, Giovannoni G, Confavreux C, *et al.* The incidence and significance of antinatalizumab antibodies. Neurology 2007; 69:1391–1403.
This study shows that immunogenicity against biologicals can be persistent or transient.

20 Baert F, Noman M, Vermeire S, *et al.* Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med 2003; 348:601–608.

21 van der Laken CJ, Voskuyl AE, Roos JC, *et al.* Imaging and serum analysis of immune complex formation of radiolabelled infliximab and antiinfliximab in responders and nonresponders to therapy for rheumatoid arthritis. Ann Rheum Dis 2007; 66:253–256.

22 Wouters D, Stapel S, Vis, *et al.* Human anti chimeric antibodies and infusion related allerci reactions in patients with rheumatoid arthritis. Allergy Clin Immunol Int: J World Allergy Org, Supplement 2 (2007).

23 Bartelds GM, Wolbink GJ, Stapel S, *et al.* High levels of human antihuman antibodies to adalimumab in a patient not responding to adalimumab treatment. Ann Rheum Dis 2006; 65:1249–1250.

24 Pijpe J, van Imhoff GW, Spijkervet FK, *et al.* Rituximab treatment in patients with primary Sjögren's syndrome: an open-label phase II study. Arthritis Rheum 2005; 52:2740–2750.

25 Maini RN, Breedveld FC, Kalden JR, *et al.* M: Therapeutic efficacy of multiple intravenous infusions of antitumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum 1998; 41:1552–1563.

26 Maini RN, Breedveld FC, Kalden, *et al.* Sustained improvement over two years in physical function, structural damage, and signs and symptoms among patients with rheumatoid arthritis treated with infliximab and methotrexate. Arthritis Rheum 2004; 50:1051–1065.

27 Bendtzen K, Geborek P, Svenson M, *et al.* Individualized monitoring of drug bioavailability and immunogenicity in rheumatoid arthritis patients treated with the tumor necrosis factor alpha inhibitor infliximab. Arthritis Rheum 2006; 54:3782–3789.

28 Haraoui B, Cameron L, Ouellet M, White B. Antiinfliximab antibodies in patients with rheumatoid arthritis who require higher doses of infliximab to achieve or maintain a clinical response. J Rheumatol 2006; 33:31–36.

29 van der Heijde D, Dijkmans B, Geusens P, *et al.* Efficacy and safety of infliximab in patients with ankylosing spondylitis: results of a randomized, placebo-controlled trial (ASSERT). Arthritis Rheum 2005; 52:582–591.

30 de Vries MK, Wolbink GJ, Stapel SO, *et al.* Decreased clinical response to infliximab in ankylosing spondylitis is correlated with antiinfliximab formation. Ann Rheum Dis 2007; 66:1252–1254.

31 van de Putte LB, Atkins C, Malaise M, *et al.* Efficacy and safety of adalimumab as monotherapy in patients with rheumatoid arthritis for whom previous disease modifying antirheumatic drug treatment has failed. Ann Rheum Dis 2004; 63:508–516.

32 Nobuyuki Miyasaka and The CHANGE Study Investigators. Clinical investigation in highly disease-affected rheumatoid arthritis patients in Japan with adalimumab applying standard and general evaluation: the CHANGE study. Mod Rheum 2008; 18:252–262.

33 Stephens S, Emtage S, Vetterlein, *et al.* Comprehensive pharmacokinetics of a humanized antibody and analysis of residual antiidiotypic responses. Immunology 1995; 85:668–674.

34 Frommel D and Allain JP, Genetic predisposition to develop factor VIII antibody in classic hemophilia. Clin Immunol. Immunopathol 1977; 8:34–38.

35 Astermark J, Oldenburg J, Pavlova A, *et al.* Polymorphisms in the IL10 but not in the IL1beta and IL4 genes are associated with inhibitor development in patients with hemophilia A. Blood 2006; 107:3167–3172.

36 van der Heijde DM, Kivitz A, Schiff MH, *et al.* Efficacy and safety of adalimumab in patients with ankylosing spondylitis: results of a multicenter, randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2006; 54:2136–2146.