Immunogenicity of biologic agents in rheumatology

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Abstract | Biologic agents have become a core component of therapeutic strategies for many inflammatory rheumatic diseases. However, perhaps reflecting the specificity and generally high affinity of biologic agents, these therapeutics have been used by rheumatologists with less consideration of their pharmacokinetics than that of conventional synthetic DMARDs. Immunogenicity was recognized as a potential limitation to the use of biologic agents at an early stage in their development, although regulatory guidance was relatively limited and assays to measure immunogenicity were less sophisticated than today. The advent of biosimilars has sparked a renewed interest in immunogenicity that has resulted in the development of increasingly sensitive assays, an enhanced appreciation of the pharmacokinetic consequences of immunogenicity and the development of comprehensive and specific guidance from regulatory authorities. As a result, rheumatologists have a greatly improved understanding of the field in general, including the factors responsible for immunogenicity, its potential clinical consequences and the implications for everyday treatment. In some specialties, immunogenicity testing is becoming a part of routine clinical management, but definitive evidence of its cost-effectiveness in rheumatology is awaited.

Phage display
A technique whereby an antibody-variable sequence is displayed on the outside of a bacteriophage that contains the DNA encoding the variable sequence, enabling the screening and selection of bacteriophages containing the genetic sequence of interest.

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Biologic agents now form part of the therapeutic armamentarium for most inflammatory rheumatic diseases. Since their early development, an emerging feature of biologic agents has been their propensity to provoke an immune response against themselves (known as immunogenicity), most notably, the generation of anti-drug antibodies (ADAs), which can have clinical consequences; for example, in the early 1990s, researchers noted that repeat courses of OKT3 (a mouse monoclonal antibody that recognizes CD3) had limited clinical efficacy because the mouse antibodies were highly immunogenic in humans. The humanization of monoclonal antibodies (and the subsequent development of 'fully human' monoclonal antibodies produced in transgenic mice carrying human immunoglobulin genes, or by phage display or single-cell cloning) have subsequently reduced the immunogenicity of biologic agents[1]. In parallel with the development of biosimilar biologic agents[2,3], ways of measuring immunogenicity have become more sophisticated and assays more sensitive over the past 10–15 years[4,5], which has led to a better understanding of immunogenicity and its consequences and a deeper knowledge of the pharmacokinetics of biologic agents[6,7]. Hence, many of the factors that provoke immunogenicity and the formation of ADAs are now well understood, although others still remain unclear.

The consequences of immunogenicity can vary and are influenced by the nature of the ADAs (for example, the antibody isotype) and the consequent immune complexes that form with the biologic agent. Although current strategies for designing monoclonal antibodies are aimed at minimizing immunogenicity via progressive humanization and innovative quality-by-design risk-minimization manufacturing methods, it still cannot be abolished completely. Thus, researchers have developed strategies to predict and lessen ADA formation. Another development has been the publication of algorithms for monitoring serum drug concentrations and ADAs in clinical practice, although the cost-effectiveness of such testing in rheumatology has not been robustly demonstrated.

In view of the growth in knowledge in the field that has occurred over the past few years, it is timely to comprehensively review the available data. In this Review, we summarize what is known about biologic agent pharmacokinetics and the factors that influence immunogenicity, including knowledge gleaned from agents used for non-rheumatic indications. We discuss the potential consequences of immunogenicity and the methods available for measuring ADAs and serum drug concentrations. We also summarize data related to the biologic agents that have been licensed for rheumatic indications, including data from studies on treatment switching,

Key points

- All biologic agents are immunogenic and many pathways influence their bioavailability, including patient-specific factors, disease-specific features and genetic background.
- The potential consequences of immunogenicity range from no clinical consequences to reduced therapeutic efficacy, infusion reactions and, rarely, serum sickness or anaphylaxis.
- Group level pharmacokinetic models have consistently shown that anti-drug antibodies (ADAs) result in decreased serum drug concentrations and reduced efficacy.
- The most important difference between available immunogenicity assays is the degree to which the assay is drug tolerant.
- Coadministration of anti-proliferative and/or immunosuppressive agents such as methotrexate decreases ADA formation and maintains serum drug concentrations via various mechanisms.
- Regular monitoring of serum drug and ADA levels has been proposed but not yet instigated into rheumatological practice, mainly owing to a lack of cost-effectiveness data.

and discuss implications for clinical practice, including the pros and cons of therapeutic drug monitoring.

Pharmacokinetics and immunogenicity

All biologic agents are immunogenic and many pathways influence their bioavailability, immunogenicity being just one of them (FIG. 1). The pharmacokinetics of monoclonal antibodies are influenced by proteolytic catabolism, target-binding capability and specific receptor-determined clearance mechanisms, including Fcγ receptor-mediated immunoglobulin clearance. IgG antibodies, including monoclonal antibody-based biologic agents, are recycled and salvaged by the neonatal Fc receptor (FcRn; also known as Brambell receptor) on vascular endothelial and reticuloendothelial system cells (such as monocytes, macrophages and dendritic cells)[8]. The structure of the monoclonal antibody itself, including its amino acid sequence, allotype, route of administration, dosing regimen and duration of treatment, can also influence both the pharmacokinetics and immunogenicity[9,10]. Another important immunogenic factor is the presence of aggregates in the therapeutic protein preparations, although modern production processes are designed to eliminate this source of immunogenicity[11].

Patient-specific factors such as a low serum albumin concentration, high BMI and/or drug target levels can also affect the clearance of biologic drugs. Concomitant administration of immunosuppressive and anti-proliferative agents such as methotrexate, azathioprine, mycophenolate mofetil and leflunomide decrease ADA formation[4] and might additionally raise biologic agent concentrations in blood[12]. Disease-specific features also affect immunogenicity. In general, lower amounts of ADAs have been reported in patients with spondyloarthritis (SpA) than in those with rheumatoid arthritis (RA) in longitudinal studies and randomized controlled trials (RCTs), despite background anti-proliferative agents being less commonly used in patients with SpA[13–15]; furthermore, the incidence of ADAs is higher in patients with more active disease. Studies have also linked variability in HLA type, HLA alleles and ethnicity to immunogenicity[16,17].

Interestingly, certain TNF inhibitors (such as infliximab, adalimumab and etanercept) seem to stabilize TNF trimers, resulting in up to 50-fold higher circulating concentrations of TNF, which plateau and stabilize during a course of treatment[18,19]. Because TNF is in a complex with its inhibitor, it is inactive, and these apparently high concentrations do not reflect disease activity. However, as early as 4 weeks into treatment, patients who later develop ADAs have lower TNF concentrations than those who do not, perhaps reflecting clearance of TNF–TNF inhibitor complexes by low affinity ADAs[18]. Furthermore, even very low concentrations of a circulating biologic agent (such as <0.1 μg/ml of adalimumab) can quantitatively neutralize TNF, suggesting a pharmacodynamic effect that might extend for many months after treatment is discontinued[18].

Early work in mouse models identified the cell-binding capacity of a monoclonal antibody therapy as a predictor of immunogenicity[20]; although it was possible to induce tolerance to antibodies that recognize soluble targets, it was difficult to tolerize to antibodies that bind to cell surface antigens[21]. The mechanisms linking the cell-binding capacity of monoclonal antibodies to immunogenicity might reflect antibody-induced cell lysis and/or enhanced presentation of immunogenic epitopes, particularly the antibody idiotype[22,23]. Although the mechanism has not been defined, following cell lysis and uptake by a phagocyte, the idiotype of the antibody might be protected from proteolysis by being bound to antigen, thereby increasing the likelihood of the presentation of immunogenic epitopes derived from the idiotype. Allotypic differences in human IgG1 antibodies might also contribute to or potentiate immunogenicity[24], although data from T cell assays and MHC-associated peptide proteomics assessing the immunogenicity of tocilizumab suggest that allotypic differences in human IgG1 are not a notable risk factor for the induction of immunogenicity with this agent[25].

In theory, humanized antibodies should be less immunogenic than chimeric antibodies owing to the presence of less non-human protein sequences in the variable region that might be recognized as foreign. Absolute evidence to support this theory is lacking as no head-to-head comparisons of an equivalent chimeric and humanized monoclonal antibody have been performed. The best evidence comes from an indirect comparison of chimeric and humanized anti-CD52 (Campath) monoclonal antibodies[26]: 15 out of 17 transplant recipients who received Campath-1G (a chimeric rat monoclonal antibody) developed ADAs, whereas none of the 12 transplant recipients who received Campath-1H (a humanized monoclonal antibody) developed ADAs. Furthermore, infliximab (the only chimeric monoclonal antibody among the five available TNF inhibitors) is more immunogenic than any of the other four TNF inhibitors[4] (TABLE 1). Indeed, an infusion reaction is more likely to occur with infliximab than with golimumab (a humanized monoclonal antibody)[27]. However, whether a humanized monoclonal antibody is more immunogenic than a fully human monoclonal antibody is unknown. Even with fully human antibodies, complementarity-determining regions (CDRs; also referred to as hypervariable regions) are still immunogenic, owing to the high variability of

Single-cell cloning
A technique whereby the antibody-encoding genetic material is extracted from a human B cell clone that produces the antibody of interest.

Idiotype
The collection of sequences (idiotopes) that form the antigen-binding site of an antibody.

Humanized antibodies
Antibodies in which the complementarity determining regions of a human antibody have been replaced with those from a mouse antibody of interest to create an antibody with the specificity of the mouse antibody in the context of a mostly human sequence.

Chimeric antibodies
Antibodies in which the variable region of a mouse antibody of interest has been genetically fused with a human constant region to create an antibody that retains the specificity of the mouse antibody in the context of a human constant region.

Fully human antibodies
Antibodies that contain only sequences derived from human genes.

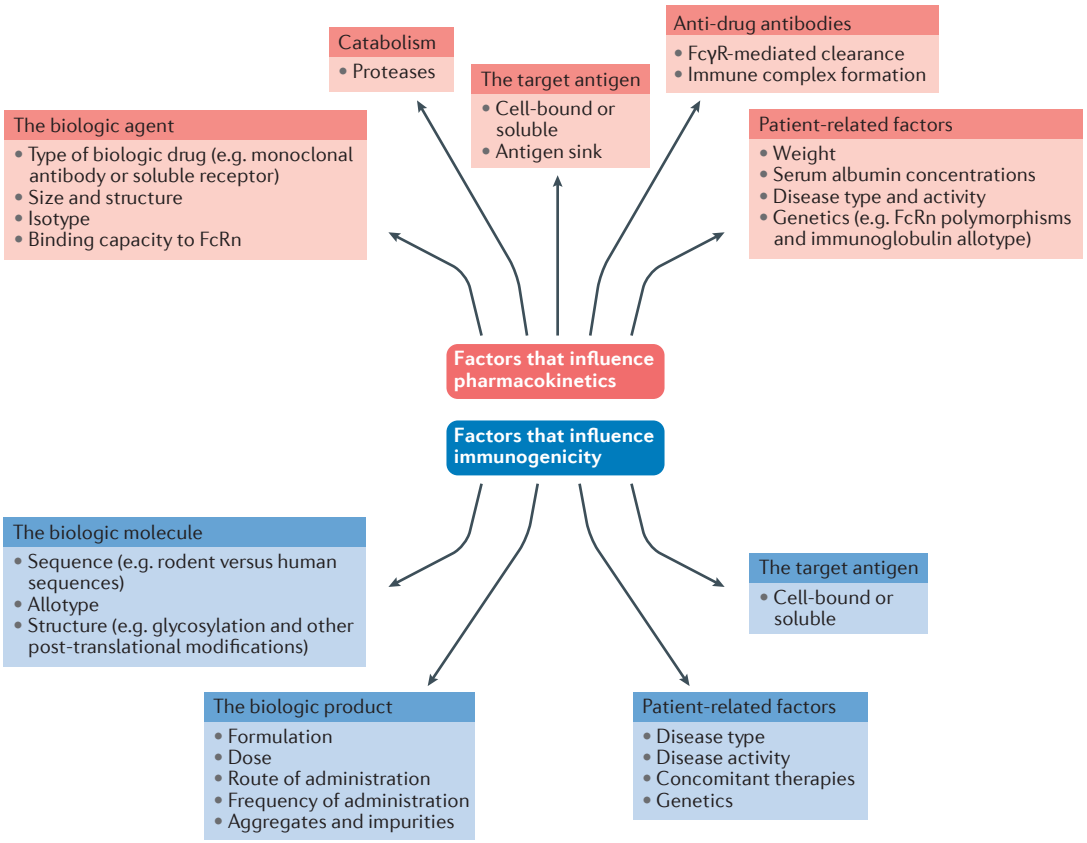


Fig. 1 | **Factors that influence the pharmacokinetics and immunogenicity of biologic agents.** Various factors can influence the pharmacokinetics of a biologic agent, including factors relating to the drug itself (the type of biologic agent, the size and structure, the isotype or the binding affinity for neonatal Fc receptor (FcRn)), the target antigen (whether the antigen is cell-bound or soluble and its level of expression), the presence of proteases that can digest the drug, the development of anti-drug antibodies (including the formation of immune complexes and accelerated clearance via Fcγ receptor (FcγR) binding) and patient-related factors (the disease being treated and disease activity, the weight of the patient, serum albumin concentrations and genetic factors). A number of factors can also influence the immunogenicity of a biologic agent, including factors relating to the drug itself (the primary sequence, the allotype and post-translational modifications such as glycosylation), the target antigen (soluble or cell-bound), the final drug product (formulation, dosing regime and route of administration or the presence of impurities or aggregates) and patient-related factors (the disease being treated and disease activity, concomitant therapies such as methotrexate and genetic factors).

CDRs following recombination events and somatic hypermutation occurring throughout life and a consequent lack of central tolerance. Whether murine CDRs are more immunogenic than human CDRs has not been formally assessed in a head-to-head comparison of a humanized and fully human monoclonal antibody. However, fully human antibodies manufactured using homologous recombination (such as golimumab, ustekinumab, secukinumab and sarilumab) are associated with lower incidences of ADAs than humanized antibodies (TABLE 1). The debate around the immunogenicity of chimeric, humanized and fully human antibodies has continued for a long time; however, the data reviewed in this article indicate that fully human antibodies are potentially the least immunogenic, an opinion shared by developers and regulatory agencies.

Pegylation and enzyme replacement

Information relevant to immunogenicity can be extrapolated from the experience of enzyme replacement therapy for haemophilia A and haemophilia B, which are caused by genetic deficiency of coagulation factors. In factor VIII and IX replacement therapy for haemophilia, the amount of cross-reactive immunological material produced by the patient determines the degree of immunogenicity and the success of the treatment[28]. Most patients with haemophilia A make some amount of factor VIII, even if the protein is non-functional, and anaphylaxis is rare, whereas the majority of patients with haemophilia B have large deletions or a minor deletion with a stop codon in the gene encoding this protein, and anaphylaxis is common[28]. Similarly, the tolerability of enzyme replacement therapy, in terms of the rate of adverse reactions such as arthralgias, injection site reactions and serum sickness, is associated with the amount of endogenous protein present, whether mutated and/or non-functional, rather than with its bioactivity[29]. Thus, the treatment of Gaucher disease (which is caused by a hereditary deficiency of the enzyme glucocerebrosidase) with recombinant glucocerebrosidases is frequently successful, owing to residual endogenous production of the enzyme. By contrast, in Pompe disease

Cross-reactive immunological material
An endogenous protein in the recipient that is immunologically similar to the replacement therapy.

(which is caused by deficiency of the lysosomal enzyme acid α-glucosidase), treatment with alglucosidase alfa (an analogue of α-glucosidase) can be complicated by nephrotic syndrome resulting from renal deposition of antigen–ADA complexes[30,31]. In mouse models of enzyme deficiencies, the use of anti-proliferative agents has been most effective if they are administered along with the first dose of enzyme replacement therapy[32].

Pegvaliase, a pegylated derivative of the enzyme phenylalanine ammonia lyase (which metabolizes phenylalanine), is approved for the treatment of phenylketonuria. One analysis of the long-term safety of pegvaliase treatment assessed the immunogenicity of pegvaliase during induction, upward titration and maintenance dosing regimens in 261 adults with phenylketonuria[33]. All patients developed ADAs to the phenylalanine ammonia lyase part of pegvaliase, the titres of which peaked at 6 months and stabilized thereafter; most patients also developed transient ADAs to the polyethylene glycol (PEG) component of pegvaliase, which peaked at 3 months and returned to baseline by 9 months. The binding of ADAs to pegvaliase led to the formation of circulating immune complexes, complement activation and hypersensitivity reactions, which most frequently occurred during early treatment and were associated with injection site reactions and arthralgias or arthritis but not with abnormalities in renal function or other serious adverse events, and was consistent with circulating immune complex-mediated type III hypersensitivity reactions. As the pegvaliase dosage increased, blood phenylalanine concentrations decreased over time, as did the amount of circulating immune complexes and complement activation. Overall, these data suggest that patients can develop tolerance to the PEG component of pegvaliase with continued regular administration, although ADAs to phenylalanine ammonia lyase can also persist.

Pegylation has also been utilized to prolong the serum half-life of biologic products with applications in rheumatology, including certolizumab pegol (a humanized $F(ab')_2$ fragment TNF inhibitor) and two pegylated uricases (pegloticase and pegadricase) used to treat patients with tophaceous gout. As uricase has not been retained in humans owing to a missense and frameshift mutation during evolution, it is very immunogenic without retained cross-reactive immunological material; the PEG conjugated to uricase is also immunogenic, perhaps reflecting broad exposure to PEGs in food additives, skin creams and personal lubricants.

In two phase III RCTs assessing the efficacy of intravenous administration of pegloticase for the treatment of chronic gout, 42% of patients were identified as 'complete responders' (maintaining serum uric acid concentrations below 6 mg/dl for more than 80% of the time)

Table 1 | **Frequency of anti-drug antibody formation in rheumatic diseases**

Biologic agent or biosimilar	RA	PsA	JIA	AS	Psoriasis	Range	Refs
Abatacept	2–20% (7)	ND	2–11% (2)	ND	ND	2–20% (9)	4
Adalimumab	0–51% (33)	0–54% (8)	6–33% (6)	8–39% (9)	0–51% (12)	0–54% (84)	4
Adalimumab biosimilar (5)[a]	31.8–43.2% (4)	ND	ND	ND	36.8–55.2% (2)	31.8–55.2% (6)	6
Certolizumab pegol	2.8–37% (7)	ND	ND	ND	21% (1)	3–37% (14)	4
Etanercept	0–13% (25)	0% (3)	0–6 % (2)	0 (4)	2–5% (5)	0–13% (37)	4
Etanercept biosimilars (2)[a]	0.3% (1)	ND	ND	ND	0% (1)	0–0.3% (2)	6
Golimumab	2–10% (11)	6% (1)	ND	0–6.4% (2)	ND	0–19% (22)	4
Infliximab[b]	8–62% (48)	15–33% (3)	26–42% (2)	6.1–69% (10)	0–41% (12)	0–83% (114)	4
Infliximab biosimilars (3)[a,b]	48.2–53.0% (3)	ND	ND	25.0% (1)	ND	22.9–53.0% (6)	6
Ixekizumab	ND	5.2–10.3% (2) with methotrexate; 8.6–12.0% (2) as monotherapy	ND	ND	ND	5.2–12.0% (2)	111
Rituximab[b]	0–21% (8)	ND	ND	ND	ND	0–21% (8)	4
Rituximab biosimilars (3)[a,b]	10.0–17.6% (5)	ND	ND	ND	ND	10.0–17.6% (5)	6
Secukinumab	ND	0–0.35% (6)	ND	0–0.69% (6)	0–1% (8)	0–1% (14)	4,108
Tocilizumab	0–16% (14)	ND	1–8% (3)	ND	ND	0–16% (17)	4
Ustekinumab	ND	8–11% (3)	ND	ND	4–8.6% (10)	1–11% (15)	4

The numbers in this table refer to percentages of patients with anti-drug antibodies across various randomized controlled trials, with the number of trials in parentheses. Adapted from REF.[4], Springer Nature Limited. AS, ankylosing spondylitis; JIA, juvenile idiopathic arthritis; ND, no data; PsA, psoriatic arthritis; RA, rheumatoid arthritis. [a]Refers to the number of biosimilars for a particular biologic agent. [b]All patients in these trials were receiving background methotrexate therapy.

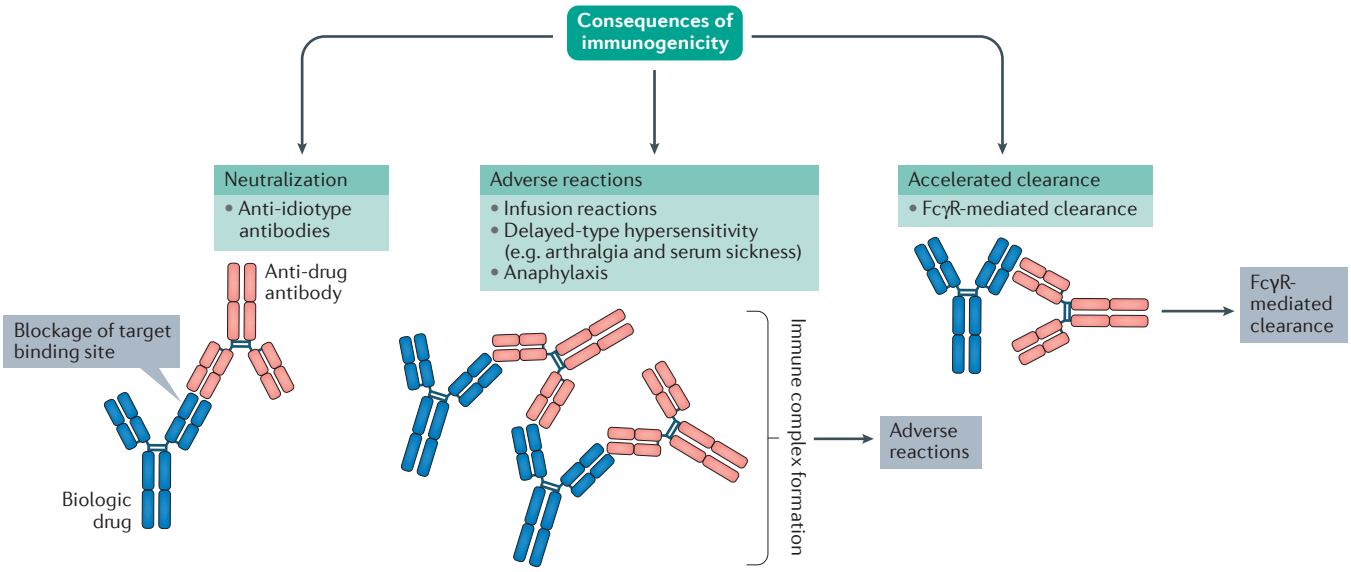


Fig. 2 | **Consequences of immunogenicity.** Anti-drug antibodies can be neutralizing if they bind at, or close to, the antigen-binding site of the therapeutic biologic agent (such as occurs with anti-idiotype antibodies). Anti-drug antibodies can also accelerate clearance of the drug via immune complex formation and binding to Fcγ receptors (FcγRs) and can lead to a variety of adverse reactions (depending on the size of the immune complex), including infusion reactions, delayed-type hypersensitivity reactions and, rarely, anaphylaxis.

at months 3 and 6 compared with 0% of those who received placebo ($P < 0.001$)[34]. Complete response was associated with complete resolution of at least one target tophus in 45% of patients who received treatment every 2 weeks[35]. Infusion reactions occurred in 26% (22 out of 85) of the patients in the treatment group, including anaphylaxis in four individuals, compared with only 5% (2 out of 43) of the patients in the placebo group; infusion reactions were predicted by serum uric acid concentrations >6.0 mg/dl before the infusion[36]. The infusion reactions were associated with the presence of ADAs, resulting in the monitoring of serum uric acid levels before each infusion being recommended in the product label which, in a post hoc analysis, would have reduced the incidence of infusion reactions to 2%[37].

High-titre ADAs to pegloticase in the phase III RCTs were associated with a loss of treatment response owing to reductions in serum drug concentrations (which thus caused serum uric acid concentrations to increase); the ADAs were not neutralizing and primarily recognized the PEG moiety, leading to accelerated drug clearance[38]. In a phase II RCT of pegloticase in 30 patients with refractory symptomatic gout, 7 of whom were organ transplant recipients, 5 of the patients had a durable response to therapy and only 1 patient developed ADAs[39]. All the patients had been receiving anti-proliferative and/or immunosuppressive drugs, including cyclosporine and tacrolimus. Subsequent case series and clinical trials of pegloticase have reported successful persistent therapeutic outcomes and even 'recapture' of lost clinical effect following initiation of anti-proliferative background therapy using methotrexate, azathioprine, mycophenolate mofetil or leflunomide[40–45].

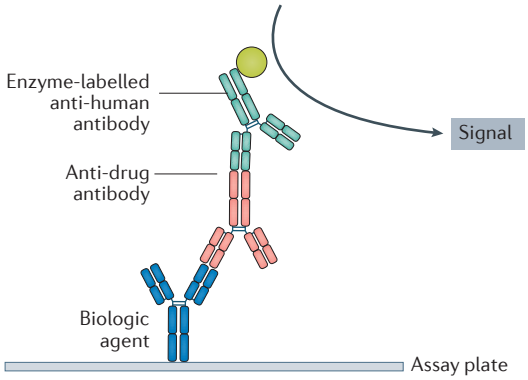
To try to mitigate immunogenic responses, the other pegylated uricase, pegadricase, is co-administered with a proprietary biodegradable nanoparticle, ImmTOR. ImmTOR encapsulates the immunomodulator rapamycin (sirolimus) to mitigate the formation of ADAs, ostensibly by delivering a tolerogenic message to dendritic cells as they are exposed to pegadricase in the spleen and liver[46]. An RCT comparing pegadricase with pegloticase is currently underway[47].

Consequences of immunogenicity

The potential consequences of immunogenicity on the pharmacokinetics and pharmacodynamics of biologic agents vary (FIG. 2). For the majority of patients, immunogenicity to biologic agents, particularly to fully human monoclonal antibody therapies, has no clinical consequences. In some individuals, ADAs are associated with reduced therapeutic efficacy, either because of immune complex formation and accelerated drug clearance and/or because of neutralizing antibodies that block monoclonal antibody binding to the epitope binding site[48]. At least some immune complexes are removed by the reticuloendothelial system in the spleen and liver[49]. ADA formation is also linked to certain adverse events following biologic agent therapy, such as injection site reactions and/or infusion reactions, the latter being more common with infliximab; less common adverse reactions to biologic agent therapy include serum sickness and anaphylaxis (which occurs rarely).

At a group level, the presence of ADAs is typically associated with lower drug concentrations and reduced efficacy and/or a secondary loss of response; but at the level of the individual, a high degree of variability exists[12]. Characteristics of the ADAs are also important. Low-affinity ADAs, which are typical in individuals with pre-existing reactivity to the drug (such as can occur with pre-existing reactivity to PEG), seem unlikely to interfere with therapy, although this theory has not been

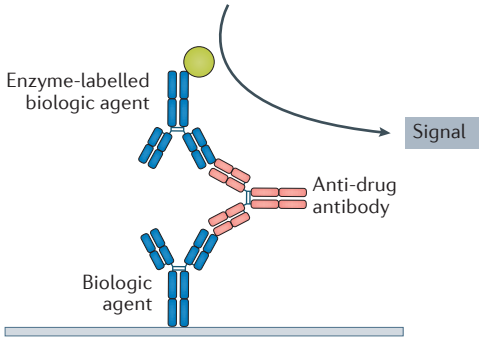
a Indirect ELISA



Strengths
- Easy to use
- High throughput
- Inexpensive
- Generic reagents and instrument

Weaknesses
- High background
- High risk of false-positive results
- Misses low-affinity antibodies
- Biologic agent immobilization can mask epitopes
- Requires species-specific secondary reagent
- Not suitable for monoclonal antibody products

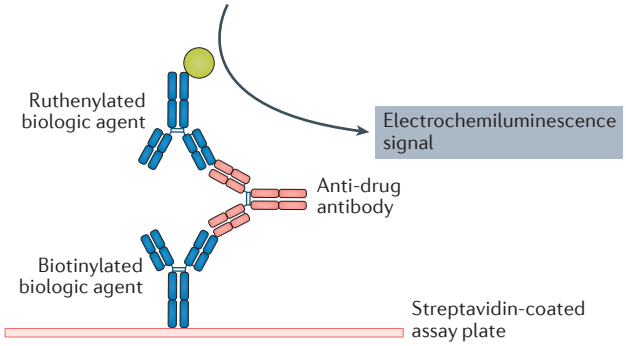
b Bridging ELISA



Strengths
- Easy to use
- High throughput
- Inexpensive
- More specific and selective than indirect ELISA
- Suitable for monoclonal antibody products
- Generic reagents and instrument

Weaknesses
- Affected by the presence of the biologic drug and other serum components (for example, anti-human Ig molecules)
- Misses low-affinity antibodies
- Might not detect IgG4 and IgM antibodies
- Antigen labelling might alter the antigen

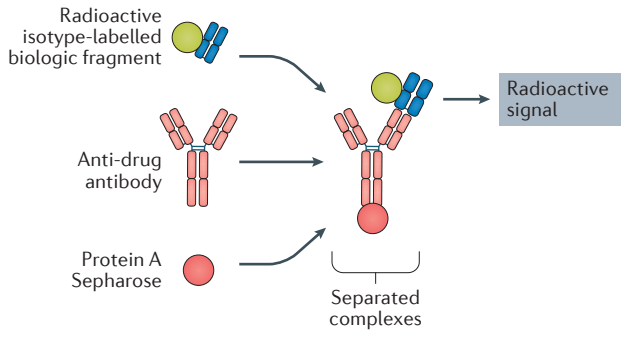
c Electrochemiluminescence assay



Strengths
- High throughput
- Large dynamic range
- Minimally affected by matrix
- High tolerance to therapeutic
- Suitable for monoclonal antibody products

Weaknesses
- Affected by the presence of the biologic drug and other serum components (for example, anti-human Ig molecules)
- Misses low-affinity antibodies
- Might not detect IgG4 antibodies
- Antigen labelling might alter the antigen
- Needs specific equipment and reagents

d Radioimmunoassay



Strengths
- Moderate throughput
- High sensitivity
- Can be specific
- Inexpensive

Weaknesses
- Requires radiolabelled antigen
- Requires special equipment and safety precautions
- Decay of radiolabel might affect the antigen stability
- Can be isotype-specific

Fig. 3 | **Immunogenicity screening assays.** The figure shows commonly used anti-drug antibody (ADA) detection immunoassay formats and their strengths and weaknesses. **a** | For indirect enzyme-linked immunosorbent assays (ELISAs), the biologic agent is coated on the assay plate, which captures any ADAs present in the sample; these antibodies are then detected by an anti-human IgG antibody conjugated to an enzyme that provides a colorimetric or chemiluminescent signal. **b** | Bridging ELISAs involve coating the biologic agent directly onto an assay plate. Following an optional acid-dissociation pretreatment step, the patient sample is added and any ADAs present are captured by the plate-bound drug. The captured ADAs are then detected using an enzyme-labelled biologic agent, so that any ADAs present must bind to two biologic agents (a plate-bound and a labelled biologic agent) to emit a signal. Other ELISA methods designed to measure ADAs make use of anti-human λ-chain-conjugated antibodies as the detector antibody instead of a labelled biologic agent. **c** | In an electrochemiluminescence immunoassay, following an acid-dissociation pretreatment step, the sample is incubated with ruthenylated and biotinylated forms of the biologic agent, which bind to any ADAs that are present. The sample is then added to a streptavidin-coated plate, which captures the ADA–biologic agent complexes. In the presence of tripropylamine and on application of an electric current, the ruthenium produces a chemiluminescent signal. **d** | In radioimmunoassays, protein A Sepharose captures the serum ADAs, which bind to radiolabelled fragments of the biologic agent, and the radioactivity of the separated complexes are measured. An important benefit of this method is that the biologic agent is in solution and has a low probability of denaturing as a result of coating. Moreover, the risk of false positives owing to binding of rheumatoid factor or non-specific antibody binding is low. Disadvantages of the radioimmunoassay method include the complexity of the test, the long incubation time and safety concerns around the use of radioactive material.

systematically studied. By contrast, repeated therapy in an individual already sensitized to the biologic agent might lead to ADAs of increasingly high affinity[50]. The amplification of pre-existing endogenous antibodies[51] presents a new challenge in the assessment of immunogenicity and its clinical relevance. In the future, it will be helpful to identify the conditions that allow ADA formation to remain limited to a low-titre, transient, IgM response with few clinical effects or that promote seroconversion.

Immunogenicity can be categorized by the functional effect of the ADAs on serum drug concentrations, that is, whether the ADAs are binding (non-neutralizing) antibodies that do not affect drug–target interactions, or neutralizing antibodies that bind to the pharmacologically active site of the biologic agent, thereby physically interfering with the ability of the drug to bind to its target[52]. The clinical importance of testing for binding ADAs or neutralizing ADAs in patients being treated with a monoclonal antibody therapy is not clear. Although neutralizing ADAs might have a direct negative effect on functional drug concentrations, the major safety concern for this type of ADA relates to enzyme replacement therapies, for which cross-reactivity to the endogenous counterpart can lead to life-threatening adverse effects[32]. However, no specific safety concerns have been reported for neutralizing ADAs to monoclonal antibody therapeutics. Nonetheless, binding ADAs might indirectly decrease drug concentrations by increasing drug clearance via immune complex formation.

As highlighted in the next section, harmonization of immunogenicity assessments is necessary. Specifically, harmonization of the type of assessments and assay strategies used for measuring the immunogenicity of a biologic product, including measurement of the immunogenicity of different biologic products in the same therapeutic class, as well as implementation of similar study and laboratory protocols to obtain comparable data, would improve our understanding of the clinical consequences of immunogenicity.

Monitoring therapeutics and ADAs

Knowledge of immunogenicity and methodologies to evaluate unwanted immune reactions have advanced considerably since the introduction of biologic therapies. The precision and sensitivity of immunogenicity assays have improved over time and will continue to do so. Consequently, the use of these new assays has highlighted a higher rate of immunogenicity than previously thought[53,54]. Clinicians should be knowledgeable about these developments and how differences between assay types might influence interpretation of the assay test results. Although the assessment of immunogenicity was of great importance during drug development, the arrival of biosimilars and the requirement to compare these drugs with their reference products in RCTs has generated new clinical information on the immunogenicity of already approved biologic therapies in rheumatology[52,55]. During this time, the technology used in immunoassay platforms has evolved, meaning that the assay platforms originally used to monitor reference products during development might now be outdated.

Information relevant for the assessment of the effects of immunogenicity on overall clinical benefit-to-risk ratios for therapeutic proteins is complex and distributed across many different sections of the regulatory dossier. Moreover, essential background information on the intrinsic immunogenic potential of the molecule, and how extrinsic factors (such as the product quality, patient variables and dose regimen) might interact to influence the clinical manifestations, is often missing. For this reason, a draft guideline on immunogenicity assessment from the EMA and guidance from the FDA on immunogenicity testing formally recommend that an "integrated summary of immunogenicity" be included in the product's regulatory dossier[56,57].

ADA testing

The detection and assessment of ADAs is complex, and results can be influenced by the assay utilized. Hence, it is important to utilize specific and approved strategies when evaluating immunological responses. Screening tests must be sensitive, specific and able to recognize all isotypes of ADAs to a given biologic agent. Platforms for assessing immunogenicity include different types of immunoassay, such as enzyme-linked immunosorbent assays (ELISAs), electrochemiluminescence immunoassays (ECLIAs) and radioimmunoassays, as well as different immunoassay formats, such as direct, indirect, bridging and competitive formats[53,54,58] (FIG. 3).

ELISAs and ECLIAs are the major platforms of choice for ADA detection because such immunoassays offer high sensitivity and throughput. Regardless of the clinical relevance of low-affinity or high-affinity ADAs, an assay should be capable of detecting a reasonable range of ADA affinities. With indirect ELISAs, ADAs are captured by the biologic agent immobilized on a plate (FIG. 3a). A major disadvantage of such assays in the setting of humanized and fully human therapeutics is high background caused by the enzyme-labelled anti-human

antibody cross-reacting with the plate-bound capture antibody. Additionally, fixation of the biologic agent to the solid surface during plate coating can alter its conformation and which epitopes are exposed, decreasing the sensitivity of the assay and leading to the potential for cross-reactivity[59]. These drawbacks have been circumvented by bridging ELISAs, in which the non-labelled biologic agent is directly immobilized on the plate in the correct orientation to allow bridging of the ADAs to the labelled biologic agent[60,61] (FIG. 3b). Disadvantages include the occurrence of false positives because of non-specific binding and loss of low-affinity ADAs during repeated washes. Sandwich versions of ELISAs are also available and are more selective and specific than either indirect or bridging formats; however, they still might lose low-affinity ADAs during washing steps[62]. Continuing improvements of immunoassays have resulted in ECLIAs, which utilize the same principles as an ELISA but use a ruthenium-conjugated protein rather than an antibody for detection, and are therefore more sensitive for detecting monoclonal antibodies[63] (FIG. 3c). Radioimmunoassays are based on high-sensitivity assay techniques to measure concentrations of antigens by the use of antibodies, or alternatively to detect antibodies that recognize a specific antigen. These assays measure the presence of an antigen with very high sensitivity. In a radioimmunoassay, the target antigen is labelled radioactively and bound to its specific antibodies. Serum is added to initiate a competitive reaction between the labelled antigens from the preparation and the unlabelled antigens from the serum for the specific antibodies (FIG. 3d). The competition for the antibodies releases a certain amount of labelled antigen, which is proportional to the ratio of labelled to unlabelled antigen. However, comparison of data in the literature seems to show that ECLIA is more sensitive than radioimmunoassay and is less affected by drug interference, with the advantage that patient and study heterogeneity is not a limiting factor for study comparisons.

The most important distinction between immunogenicity assays is the extent to which the assays are drug-tolerant; in other words, how sensitive an assay is to the presence of the biologic agent in the serum, which, when present in equivalent concentrations to ADAs, causes the formation of immune complexes[64]. The concentration of the biologic agent in the sample needed to interfere with ADA detection depends on the amount of ADA present in the patient sample, meaning that the drug tolerance of an assay will be higher for serum with high ADA titres and lower for serum with low ADA titres. To detect ADAs with high confidence, assays must have high specificity and sensitivity. Moreover, it is important to minimize drug interference in an assay, which can be achieved by several strategies, such as sample pretreatment, the use of drug-tolerant assays and the use of competing antibodies. For example, in bridging ELISAs, ADAs link non-labelled biologic agent to labelled biologic agent; thus, immune complex formation precludes recognition of the bridging moiety and can lead to underestimation of immunogenicity. To overcome this technical weakness, drug-tolerant assays have been developed by adding an acidic or basic pretreatment step designed to dissociate ADA–drug complexes in serum samples[65]. Other technical advances that have been used to increase the drug tolerance of assays include affinity capture elution and the use of nanoparticles or magnetic beads. Data from such assays consistently show that low-affinity ADAs are detectable at 2–4 weeks after the initial biologic agent dosing and that the majority of ADAs are evident within 12–24 weeks[66].

Whether drug-tolerant assays are more useful than other assays in clinical practice is a subject of debate. These assays detect ADAs that decrease drug serum concentrations in the patient, but also detect low-affinity antibodies that do not cause clinically relevant changes in the pharmacokinetics of the drug. Furthermore, large ADA–drug complexes are eliminated rapidly from the circulation, which can lead to immunogenicity being underestimated[62]. By contrast, drug-sensitive assays typically only reveal ADAs when serum trough concentrations are below clinically relevant concentrations. Therefore, clinical judgements made on the basis of drug-tolerant assay results must be carefully assessed, given that the strong associations between immunogenicity and clinical effects were mostly established using drug-sensitive assays[67–69].

Irrespective of the technique used to detect ADAs, assay validation parameters should include cut-off points, sensitivity, drug tolerance, specificity, precision, dilution range of the serum and reproducibility[62,70]. In the absence of reference standards, these assays are simply quasi-quantitative. As a consequence, to correctly interpret ADA test results, the dose, timing of administration and serum drug concentrations should be determined concomitantly with immunogenicity. In practical pharmacokinetic terms, the assessment of clinical immunogenicity requires collecting samples at the end of the drug elimination phase (that is, when the drug is at its lowest concentration) immediately before the next administration, to avoid drug interference in the assay. Repeated testing is useful for determining whether the ADAs are transient. If necessary, a positive test result should be confirmed by incorporating an excess of biologic agent into the assay, which will reduce the signal of a truly positive ADA result. The detection of ADAs is typically followed by assessments of the magnitude (titre) of the ADA response, especially in late-stage clinical studies. ADA titres provide more useful information for the interpretation of ADA data and for determining relationships with clinical outcomes than mass concentrations. Hence, ADA titres are usually determined by running positive samples in serial dilution and reporting the titre as the reciprocal of the last dilution at which the sample scores are negative. Samples verified as ADA-positive might also be subsequently tested for the presence of neutralizing ADAs using cell-based bioassays or competitive ligand-binding assays. Cell-based bioassays, which monitor the function of the biologic agent in the presence of neutralizing ADAs, are recommended by the FDA. However, cell-based approaches can be laborious and difficult to develop, despite the provision of validation guidelines provided by the FDA and EMA[59,60].

Table 2 | **Characteristics of TNF inhibitors**

Characteristic	Adalimumab	Certolizumab pegol	Etanercept	Golimumab	Infliximab
Molecular structure	Fully human IgG1κ monoclonal antibody	Pegylated F(ab´)^2 fragment of humanized IgG1κ monoclonal antibody	Fusion protein of a human TNFR2 and IgG1 Fc region	Fully human monoclonal antibody	Chimeric (mouse and human) monoclonal IgG1κ antibody
Binding specificity	TNF	TNF	TNF and lymphotoxin	TNF	TNF
Anti-nuclear antibody induction	+++	+	+	++	++
FcγR binding	++	–	+/–	ND	++
Transmembrane TNF neutralization	+++	+++	++	+++	+++
Reverse signalling (apoptosis)	+++	–	+/–	+++	+++
Reverse signalling (cytokine suppression)	+++	+++	++	+++	+++
Antibody-dependent cytotoxicity[a]	+++	–	+/–	+++	+++
Complement-dependent cytotoxicity[a]	+++	–	+/–	+++	+++
Associated with lupus-like syndrome	Yes	Yes	Yes	Yes	Yes
Associated with demyelination or neuropathies	Yes	Yes	Yes	Yes	Yes

Adapted with permission from REF.[128], Elsevier. FcγR, Fcγ receptor; ND, no data; TNFR2, TNF receptor 2. [a]Examined under in vitro conditions.

Therapeutic drug monitoring

Population pharmacokinetic models have consistently shown that ADAs that recognize TNF inhibitors can increase the clearance rate of the drug, resulting in decreased serum drug concentrations, as occurs with infliximab, adalimumab, golimumab and certolizumab pegol[71,72]. Therefore, the pharmacokinetics of the therapeutic protein can also be used as a marker of immunogenicity. Therapeutic drug monitoring requires a different methodology to immunogenicity assays but similarly lacks a single standard technique or algorithm. Differences between pharmacokinetics data are not caused by a lack of correlation between results obtained using different methodologies, as clinical decisions are often similar regardless of the assay used. However, 20–30% of therapeutic drug monitoring results are potentially incorrect because of differences in how the cut-off levels of the assays are determined[73]. Therefore, more evidence from RCTs is needed during the development of biologic agents to identify and optimize the use of immunogenicity assays in clinical practice. To improve therapeutic decision-making, the same assays and cut-off values should be used during the follow-up of each individual patient.

In terms of implementing therapeutic drug monitoring and immunogenicity testing in clinical practice, usually >20 samples a day are required to enable the laboratory to make results clinically available in a cost-effective matter. As a result, the clinician frequently only has the results of the last test just in time for the next scheduled infusion. To surmount this disadvantage, rapid point-of-care tests for measuring serum concentrations of TNF inhibitors are becoming increasingly available[73]. Quantitative and qualitative validation of these tests against conventional ELISAs has been successful[72]. Such rapid testing offers many advantages, such as enabling testing during outpatient visits for patients who do not respond to therapy and who need to be monitored by a nurse, physician or researcher before their next infusion. Because of the rapidity of obtaining the test results (results are typically obtained within 15–20 min), treatment can be adjusted immediately rather than at the subsequent infusion (which typically occurs 6–8 weeks later)[63].

Immunogenicity of biologic therapies

In this section, we review published immunogenicity data for biologic agents that are licensed or approved for use for inflammatory arthritis (including RA, psoriatic arthritis (PsA) and ankylosing spondylitis).

TNF inhibitors

Infliximab. Being chimeric, infliximab is the most immunogenic of the TNF inhibitors[4] (TABLE 2). The presence of ADAs to infliximab is generally associated with reduced serum infliximab concentrations, decreased pharmacodynamic effects and clinical responses and a greater risk of infusion reactions[4]. ADAs are formed to the mouse portion of the monoclonal antibody, which includes the epitope binding region, and are generally of the IgG, IgA or IgM isotype or, less commonly, the IgE isotype[74].

The formation of large, irregularly shaped ADA immune complexes occurs in patients with high serum concentrations of both infliximab and ADAs, as can happen

during and following intravenous administration[49]. Following infusions, peak serum concentrations of infliximab can reach as high as 100–150 mg/ml, approximately ~1% of the total serum IgG antibodies; the presence of equimolecular concentrations of the drug and ADAs can lead to the rapid formation of soluble IgG complexes[49]. Individual anti-infliximab antibody clones have different propensities to form dimers, tetramers, hexamers and even larger complexes in vitro[49]. Large immune complexes are rapidly internalized by macrophages and preferentially cleared in vivo, leaving only dimer complexes in the circulation. Large disorganized complexes, especially those larger than hexamers, activate the complement cascade and result in subsequent infusion reactions, which therefore represent a type III hypersensitivity reaction (immune-complex mediated) rather than a type I IgE hypersensitivity reaction, which is consistent with the rarity of detection of IgE ADAs[28,49].

ADA formation is lower in patients with RA receiving higher doses of infliximab than in patients receiving lower doses; induction regimens and background therapy with methotrexate and/or leflunomide also reduce the incidence of ADAs in patients with RA[60]. ADAs to infliximab or its biosimilar CT-P13 occur at a lower rate in those with SpA than in those with RA, even in the absence of background therapy[13,15]. ADA titres also increase with the duration of therapy.

As related in an earlier section, some ADA responses are conventional, T cell-dependent, immune reactions[22]. Researchers have identified various immunogenic T cell epitopes in the variable chain regions of infliximab and rituximab by deriving $CD4^+$ T cell lines generated from 15 healthy individuals[75]. Six of the nine T cell epitopes identified could stimulate peripheral blood mononuclear cells from patients sensitized against infliximab or rituximab, promoting the secretion of a diverse range of cytokines. Thus, the identification of neo-epitopes and their MHC binding capabilities might, in some cases, predict the immunogenicity of therapeutic monoclonal antibodies. Removing such epitopes from the amino acid sequence of the therapeutic monoclonal antibody could decrease its immunogenicity; however, this approach would require an entirely new clinical development programme as the modified biologic would be considered a new monoclonal antibody therapy rather than a biosimilar.

Immunogenicity data are also available for three of the infliximab biosimilars[6] (TABLE 1). On the basis of more sensitive assays, the incidence of ADAs in patients with RA receiving background methotrexate approximates 50%, the majority of which are neutralizing. Positivity for ADAs is associated with lower serum drug concentrations, reduced clinical responses and infusion reactions. Epitope recognition was similar between biosimilars and reference product, showing a similar antigenic presentation. Potentially immunogenic epitopes are mainly present in the variable light chain and heavy chain but are also present in the Fc domain.

Adalimumab. Adalimumab is a fully humanized anti-TNF antibody that was developed using phage display substitution, a method that was the subject of the Nobel prize for chemistry in 2018 (REFS[76,77]). Even with humanization, heavy and light variable chain amino acid sequences adjacent to the epitope binding site within the CDR of the monoclonal antibody are broadly immunogenic in healthy volunteers as well as in patients with autoimmune diseases. This immunogenicity has been confirmed by prominent $CD4^+$ T cell responses to adalimumab in samples from around 100 healthy individuals[78].

ADAs to adalimumab are predominantly neutralizing ADAs of the IgG1 or IgG4 isotype that circulate as small dimeric immune complexes. These ADAs have been extensively studied, particularly in Dutch cohorts of patients with RA or PsA[74,79–85]. The majority of patients develop ADAs within the first 28 weeks of treatment; high titres are associated with low or undetectable serum drug concentrations, reduced clinical responses and, less commonly, injection site reactions. ADA levels increase with longer duration of therapy. Induction regimens (in patients with Crohn's disease) and the use of background therapy with methotrexate or other anti-proliferative agents maintain adalimumab blood concentrations and decrease ADA formation[18,86,87]. Coadministration of methotrexate prolongs the half-life of adalimumab by 40–50%[88]; an effect that is dose-dependent[89], distinct from its effects on immunogenicity, and presumably caused by inhibition of Fc-mediated clearance mechanisms[66] or increased FcRn expression in tissues[90,91]. Methotrexate does not have a similar effect on prolonging the half-life of the TNF inhibitors etanercept or certolizumab pegol. Methotrexate also reduces serum TNF concentrations and, owing to reduced target-mediated drug disposition, contributes to increased TNF inhibitor concentrations and improved clinical responses[91]. However, given the high quantity of TNF inhibitor compared with TNF, a reduction in target-mediated drug disposition does seem to be a plausible explanation. Another hypothesis is that methotrexate suppresses early B cell and T cell responses towards the biologic agent, leading to immune modulation that is dependent, in part, on red blood cell methotrexate polyglutamate concentrations and thus the dose and duration of methotrexate administration[92,93]. To date, the CONCERTO trial[89] is probably the RCT that has best addressed the appropriate dosing of methotrexate with adalimumab, although the results were confounded as all patients had been receiving methotrexate before enrolment. Given the long half-life of red blood cell methotrexate polyglutamate concentrations, the effects of changes in drug doses reported in the trial might have been delayed.

Serum concentrations of 5–8 μg/l adalimumab are associated with optimal clinical benefit in patients with RA or PsA, although a threshold serum adalimumab concentration and a predictor of remission could not be identified in patients with Crohn's disease[6]. Immunogenicity data are also available for the six adalimumab biosimilars[3] (TABLE 1). ADAs to the biosimilars are consistently detected in approximately 40–50% of patients with RA receiving background methotrexate and in 50–60% of patients with psoriasis receiving biosimilar monotherapy. The majority of these ADAs

Target-mediated drug disposition
When the binding of a drug to its target affects the pharmacokinetics of the drug.

(50–100%) are neutralizing ADAs, although results can vary depending on the type of assay utilized.

Golimumab. Golimumab is a fully human anti-TNF monoclonal antibody that was produced using homologous recombination in genetically modified mice. Overall, the incidence of ADAs to this biologic agent is low, typically ranging from 2% to 19%[4]. Nonetheless, as with adalimumab, the presence of ADAs is associated with low or undetectable serum drug concentrations, reduced clinical responses and injection site reactions[94]. Immunogenicity is lower with intravenous administration than with subcutaneous administration, and the use of background methotrexate improves serum concentrations of the drug. For example, following subcutaneous administration of golimumab, ADAs were detected in 5 out of 33 patients with RA compared with 1 out of 43 patients with ankylosing spondylitis when tested at 24 weeks[94].

In the AWARE trial, an observational study comparing golimumab with infliximab treatment in 1,270 patients with RA, 14.2% of the patients receiving infliximab and 3.9% of the patients receiving golimumab had infusion reactions. Rates of ADAs were higher in those receiving infliximab than in those receiving golimumab, irrespective of prior biologic exposure or methotrexate use[27].

Etanercept. Etanercept is a fusion protein of the p75 component of soluble TNF receptor 2 (TNFR2) and the IgG1 Fc region. The incidence of ADAs to etanercept is low, in part because many commercial assays are designed to assess the binding of ADAs to epitope-binding regions, which will not detect anti-linker-region ADAs[4]. A similar agent, lenercept, which is a fusion protein of the p55 component of soluble TNFR1 and the IgG1 Fc region, causes the formation of antibodies to two major linear epitopes located in close proximity to the linker region that can, with epitope spreading, yield anti-Fc region ADAs, which are associated with serum sickness; by contrast, no inhibition of epitope-binding regions was reported[95]. There has been at least one case of serum sickness associated with administration of etanercept to an adult patient with juvenile idiopathic arthritis (W. H. Robinson, personal communication). Immunogenicity data are also available for three etanercept biosimilars (TABLE 1). Overall, the incidence of ADAs is low, <10%, and all ADAs are non-neutralizing (some of which are transient); however, the association between ADAs and pharmacokinetics has not been investigated[6,96].

Lower serum drug concentrations are associated with the presence of ADAs and diminished clinical responses. In one study looking at the relationship between etanercept concentrations and clinical responses in patients with RA, the patients with lower serum concentrations of etanercept were predominantly women, had a higher BMI and glomerular filtration rate and were receiving lower doses of methotrexate than those patients with higher serum concentrations of the biologic agent[97]. However, no ADAs were detectable in the sera of these patients, which might otherwise have explained these findings. In another study involving 186 patients with RA, circulating concentrations of TNF increased in the patients following the administration of etanercept, similar to the effects seen with adalimumab[20]. Notably, in RCTs, etanercept combined with methotrexate therapy is more effective than etanercept monotherapy in patients with RA, regardless of the dose of methotrexate administered.

Certolizumab pegol. Certolizumab pegol is a F(ab′)$_2$ fragment of a humanized anti-TNF antibody that is conjugated to PEG. In various RCTs of this drug in patients with RA or psoriasis, 3–37% of the patients developed ADAs (TABLE 1); the majority of the ADAs were neutralizing and were associated with lower serum drug concentrations and reduced efficacy[4,98]. In a study of 115 patients, ADA formation correlated inversely with serum drug concentrations (both measured in random samples rather than in trough blood samples) and higher concentrations of the biologic agent correlated with a good treatment response. In a smaller study of 40 patients with RA, 65% of the patients developed ADAs, but the presence of these antibodies did not seem to influence the circulating drug concentrations in these individuals (measured in trough serum samples). The presence of ADAs was associated with a reduction in drug concentrations over time; nevertheless, certolizumab pegol concentrations remain high in most ADA-positive patients. Furthermore, ex vivo, the TNF neutralization capacity of the patients' blood correlated with their serum drug concentrations but not with the formation of ADAs, potentially reflecting the presence of ADAs that recognize the PEG component of the drug[99]. Use of an initial loading dose of certolizumab pegol and concurrent methotrexate therapy helped to mitigate immunogenicity, regardless of the dose of methotrexate used.

Rituximab

As a B cell-depleting, chimeric anti-CD20 monoclonal antibody, the immunogenicity of rituximab is underestimated[4]. This therapy is administered intermittently, and repeated courses of rituximab, particularly in patients with autoimmune diseases such as RA, systemic lupus erythematosus and anti-neutrophil cytoplasmic antibody-associated vasculitis, can result in loss of response in some individuals, which can be recaptured using a humanized or fully human anti-CD20 monoclonal antibody[100]. As discussed earlier for infliximab, epitope mapping studies have revealed potentially immunogenic T cell epitopes in rituximab[75].

Background therapy with methotrexate and other anti-proliferative agents is associated with a lower incidence of ADAs to rituximab and a longer efficacy of treatment. However, studies of the effect of differing dose regimens on the immunogenicity of rituximab are lacking. Data are also available for the three rituximab biosimilars[3]; in RCTs of these biosimilars in RA, 0–21% of patients had ADAs following the second course of therapy (TABLE 1).

Abatacept

Abatacept is a cytotoxic T lymphocyte protein 4 (CTLA4)–Fc fusion protein, designed to inhibit T cell activation. The immunogenicity of abatacept has been

extensively studied in various RCTs in patients with RA, including studies comparing intravenous with subcutaneous administration, as well as studies of the effect of switching from intravenous to subcutaneous therapy or the effect of discontinuation and reinstitution of subcutaneous treatment (reviewed in detail elsewhere)[4]. As with etanercept, the immunogenic portion of abatacept is the linker between the CTLA4 extracellular domain and the IgG1 Fc region. In all the switching studies, <5% of the patients had ADAs following either intravenous or subcutaneous administration, switching or discontinuation and restart of therapy, and all of the antibodies were non-neutralizing.

IL-6 inhibitors

Tocilizumab. Tocilizumab is a humanized monoclonal antibody to the soluble IL-6 receptor (sIL-6R). As with abatacept, the immunogenicity of tocilizumab has been studied in RCTs comparing intravenous administration and subcutaneous administration in patients with RA[1,101]. The immunogenicity of subcutaneous and intravenous tocilizumab was similar when tested using a non-drug-tolerant assay with moderate sensitivity: 69 (1.2%) of the 5,875 patients treated with intravenous tocilizumab and 47 (1.5%) of the 3,099 treated with subcutaneous tocilizumab were ADA-positive; the majority of ADAs were neutralizing[101]. Anaphylaxis events can occur with intravenous therapy and were reported in 0.1% of patients with RA (3 of 2,644) in the 24-week results of RCTs of this therapy, and in 0.2% of patients (8 of 4,009), generally during the second to fourth infusions, in a study looking at long-term exposure[102]. Anaphylaxis also occurred in 1 patient (out of 56) in a trial of tocilizumab in patients with systemic juvenile idiopathic arthritis[103]. Furthermore, 4% of the patients receiving intravenous tocilizumab had infusion reactions and 10% of the patients receiving subcutaneous tocilizumab had injection site reactions[101].

Notably, drug reaction with eosinophilia and systemic symptoms syndrome was reported in a patient with adult-onset Still's disease following administration of 8 mg/kg intravenous tocilizumab[104]. The rash produced by the drug differed from the original rash caused by the disease, and a biopsy confirmed the presence of a lymphocytic and eosinophilic perivascular infiltrate, which was associated with a high peripheral eosinophil count and elevated liver function tests.

Sarilumab. Sarilumab is a fully human monoclonal antibody to sIL-6R, produced using homologous recombination. Using a sensitive assay (an ECLIA that included an acid dissociation step), ADAs were assessed in 132 patients with RA who were randomly assigned to receive 150 mg ($n = 65$) or 200 mg ($n = 67$) sarilumab every 2 weeks[105]. Persistent ADAs were detected in 12.3% and 6.1% of individuals receiving the 150 mg and 200 mg doses, respectively, of which 6.1% and 3.0% were neutralizing ADAs. A single hypersensitivity event of rash was reported and no incident anaphylaxis, and the presence of ADAs affected neither the efficacy nor the safety of the drug, which produced similar responses in ADA-positive and ADA-negative patients.

IL-12–IL-23 inhibitor

Ustekinumab is a human IgG1 monoclonal antibody to the p40 subunit of IL-12 and IL-23 that is approved for the treatment of psoriasis, PsA and Crohn's disease. Immunogenicity data are available from RCTs in psoriasis and PsA[1], as well as from a prospective observational study of ustekinumab in 76 patients with plaque psoriasis (in which serum concentrations of ADAs and ustekinumab were measured by radioimmunoassay and ELISA, respectively)[106]. In the latter study, after a mean of 13 months of treatment, ADAs were detectable in 6.5% of the patients, the presence of which were associated with significantly lower serum drug concentrations (0.01 mg/l versus 0.2 mg/l; $P < 0.001$) and a reduced treatment response (as assessed by a 50% reduction in the psoriasis area and severity index score; 0% versus 69%; $P = 0.004$). The percentages of ADA-positive patients were similar among those with prior exposure to adalimumab with and without anti-adalimumab antibodies (14.3% versus 12.5%; $P = 1.00$).

Researchers have compared and validated different measurement approaches for the assessment of ustekinumab immunogenicity, following the recommendations of the EMA and FDA[107]; in this assessment, a newly developed ELISA-based acidification assay for detecting neutralizing ADAs was compared with surface plasmon resonance, a conventional ELISA and cell-based neutralization assays. The detection of ADAs was increased after the acidification step, indicating the release of ustekinumab from binding sites owing to the presence of neutralizing ADAs.

IL-17A inhibitors

Secukinumab. Secukinumab is a fully human monoclonal antibody that recognizes IL-17A and is approved for the treatment of psoriasis, PsA and SpA[1]. In RCTs, researchers have used ECLIAs to assess the immunogenicity of secukinumab (administered as monthly subcutaneous infusions with or without intravenous or subcutaneous loading doses) in PsA (the FUTURE 1–3 RCTs) and in SpA (the MEASURE 1–4 RCTs) at baseline and at weeks 16, 24 and 52 (REF.[108]). In the treatment groups, ADAs were detectable in 0.35% (5 of 1,414) of the patients with PsA and 0.69% (8 of 1,164) of the patients with SpA over 52 weeks; 2 of the 5 ADA-positive patients with PsA and 1 of the 8 ADA-positive patients with SpA had received concurrent methotrexate therapy. Only one of the patients had neutralizing ADAs, and the presence of ADAs was not associated with changes in serum drug concentrations, loss of efficacy or adverse events. Data from MHC-associated peptide proteomics analysis and T cell activation assays suggest that secukinumab is comparable to other fully human monoclonal antibodies with low immunogenicity with regard to the types of potential T cell epitopes and T cell response rates[109].

Ixekizumab. Ixekizumab is a humanized monoclonal antibody to IL-17A that is approved for the treatment of psoriasis, PsA and SpA. ADAs have been detected using a drug-tolerant affinity capture elution approach, in which ADA-positive patients were divided into

negative, low (<1:160), moderate (≥1:160 to <1:1,280) and high (≥1:1,280) titre groups[110]. In 385 patients with psoriasis who were treated for 60 weeks in a phase III RCT, 17.4% had detectable ADAs, only 3.5% of which were neutralizing ADAs. Some preliminary immunogenicity data on ixekizumab in PsA is available from the SPIRIT-P1 RCT (biologic-naive patients) and the SPIRIT-P2 RCT (patients who have an inadequate response or intolerance to TNF inhibition), in which patients received a 160-mg loading dose subcutaneously followed by 80 mg ixekizumab every 2 or 4 weeks[111]. Of the 223 patients from both RCTs being treated concomitantly with ixekizumab and methotrexate, ADAs were detectable in 11 (10.3%) and 6 (5.2%) of those on 4-week or 2-week dosing regimens, respectively, and of the 222 patients receiving ixekizumab monotherapy, ADAs were detectable in 13 (12%) and 9 (8.6%) of those on 4-week or 2-week dosing regimens, respectively[111]. As in psoriasis, the majority of the ADAs were present at low titres, and some were neutralizing, but ADA positivity did not have an effect on the long-term efficacy of the drug.

A sensitive T cell assay format has also been used to determine reactivity to secukinumab, ixekizumab and adalimumab in 16 healthy individuals[112]. Monocyte-derived dendritic cells from individuals with the most common HLA-DR alleles that occur in the ethnically mixed European population were incubated with either the individual monoclonal antibodies or with keyhole limpet haemocyanin (KLH) as a positive control. $CD4^+$ T cell lines were then generated in vitro by co-culture with the dendritic cells, the antigen specificity of the T cell lines tested by a type I interferon ELISpot assay and the mean frequency of antigen-specific cells per million donor cells determined. Responses were detected to KLH in all samples, whereas only 1 individual responded to secukinumab, 9 responded to ixekizumab and 9 responded to adalimumab, reflecting the lower immunogenicity of secukinumab compared with ixekizumab or adalimumab.

Switching studies

Several open label studies have investigated the effects of switching biologic therapies in patients with rheumatic diseases who do not respond, or who respond poorly, to a TNF inhibitor; these patients might either be switched to another TNF inhibitor or to a different class of biologic therapy, such as abatacept or rituximab. The results of the RESTART trial ($n = 197$) confirmed that patients who do not respond to either adalimumab or etanercept might respond to infliximab, with 52% of the switched patients achieving a EULAR clinical response at week 26 (REF.[113]). In a different cohort study investigating a switch to adalimumab in patients who did not respond to infliximab therapy ($n = 235$), patients with ADAs to infliximab developed non-cross-reactive ADAs to adalimumab more often than patients without ADAs to infliximab (27% versus 18%; $P = 0.039$); however, there was no difference in the changes in the 28-joint disease activity score between the two groups[79]. Thus, more ADA-positive individuals who switch therapy develop non-cross-reactive ADAs to a second or third TNF inhibitor than ADA-negative individuals who switch; however, many individuals with a secondary inadequate response show no evidence of immunogenicity. In a separate study, 89 individuals with a secondary inadequate response to adalimumab or infliximab were switched to etanercept and compared with 203 TNF inhibitor-naive patients[114]. There was no difference in responses between TNF inhibitor-naive and ADA-positive individuals who switched medication, whereas poorer responses were seen in those who switched medication and were ADA-negative, suggesting that inadequate responses to therapy in this latter group reflected synovitis that was no longer TNF dependent.

For 'non-medical' drug switching, for example, switching from intravenous to subcutaneous administration of the same biologic agent for convenience and/or cost reasons, the therapeutic responses before and after switching are usually comparable[115]. Non-medical switching to biosimilars has been reviewed extensively elsewhere[6] and is not a topic for this manuscript. As no biosimilar has been approved in the USA as an interchangeable product (a regulatory designation only available in the USA), switching between a reference product and a biosimilar is not currently relevant to clinical practice in the USA.

Clinical practice implications

Given the immunogenicity of TNF inhibitors, and its therapeutic consequences, researchers have advocated monitoring serum drug and ADA levels[116,117], although the cost-effectiveness of this practice has not yet been as robustly demonstrated for rheumatic diseases as it has for inflammatory bowel disease[118–120]. In theory, measurements of circulating drug concentrations could enable rheumatologists to personalize dosing, avoiding both under-exposure to the drug, which might reduce treatment efficacy, and over-exposure to the drug, which might increase the risk of adverse events, such as infections. Combined with ADA measurements, drug concentration measurements might also be helpful when assessing non-response to therapy (FIG. 4).

At a population level, an optimal blood concentration of a biologic agent theoretically exists that maintains the patients in sustained remission without leading to over-exposure to the drug. For example, with adalimumab, a trough concentration of 51 g/ml might be optimal[121]. Using this knowledge, it might then be possible to lengthen the intervals between doses for patients with high trough concentrations. Similar approaches could be developed for other biologic agents. Conversely, if patients fail to respond to a therapy, then knowledge of drug concentrations and ADAs can also be helpful. In individuals who exhibit a primary non-response to a therapy, a serum drug concentration measurement within the therapeutic range could suggest the need to switch to another class of drug, whereas low serum drug concentrations could suggest that a higher dose is necessary. Similar rules can apply for individuals with a secondary non-response to therapy, except that the presence of ADAs alongside low serum drug concentrations might suggest that an alternative therapy from the same biologic class be

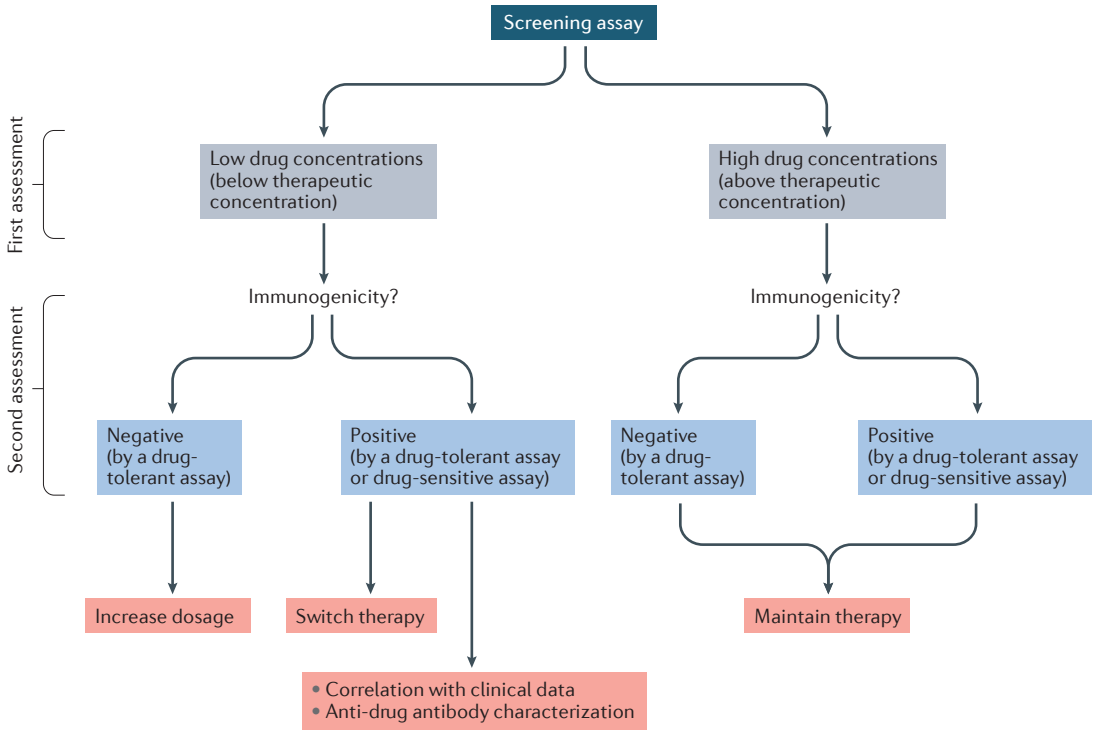


Fig. 4 | **Therapeutic drug monitoring strategies.** A potential therapeutic drug monitoring decision algorithm that integrates information regarding serum drug concentrations and immunogenic responses and that could be used in the assessment of patients with rheumatoid arthritis being treated with TNF inhibitors. The algorithm also illustrates how the assays can potentially help to guide treatment strategy. For example, if loss of efficacy of an anti-TNF monoclonal antibody is associated with the development of anti-drug antibodies, then a different TNF inhibitor might be effective. However, if loss of efficacy is not associated with anti-drug antibody development, then the best strategy might be to switch to a different therapeutic class.

selected, whereas low serum drug concentrations in the absence of ADAs might suggest poor adherence, assuming that the drug was previously effective[89,93]. Despite these theoretical benefits, preliminary results from the NOR-DRUM study revealed no clinical benefits of therapeutic drug monitoring in patients initiating infliximab therapy across a variety of inflammatory diseases[122]; no clinical differences were observed between the therapeutic drug monitoring group (consisting of individualized therapy with infliximab according to serum drug concentrations and ADA status) or the control group (administration of infliximab without knowledge of the serum drug concentrations or ADA status) after 30 weeks of treatment. However, the study did not specify how often the dose was adjusted in the therapeutic drug monitoring group, which would have been useful to know, particularly for those patients with sub-optimal serum drug concentrations. In terms of adverse effects, the development of infusion reactions with infliximab in the presence of ADAs should prompt a change of treatment, potentially to another TNF inhibitor. However, only a minority of patients with ADAs develop infusion reactions. Injection site reactions might reflect immunogenicity but can also be attributed to the formulation of the agent in use.

Without therapeutic drug monitoring, the decision to switch to a different therapy following a primary or secondary inadequate response depends on the clinician's inclination and the preference of the hospital. Therapeutic drug monitoring could improve this procedure by identifying subgroups of patients who might profit from switching to either a second TNF inhibitor or to a biologic of a different class. For example, loss of clinical response to a first TNF inhibitor in the absence of ADAs is predictive of a potential lack of response if switched to a second TNF inhibitor[123]. However, in a series of 137 patients with RA, neither the ADA status nor the serum drug concentrations were predictive of subsequent responses to TNF inhibitors or to other classes of biologic agents in patients who were not responsive to adalimumab therapy[124]. Notably, this study was a retrospective analysis of patient data rather than a prospective trial, and used random samples rather than trough blood samples to measure drug concentrations and ADAs. Importantly, clinical monitoring is adequate on its own in rheumatology, unlike other specialties, which might require additional invasive tests.

Although a number of algorithms have been developed for therapeutic drug monitoring of biologic agents, a major remaining question relates to cost-effectiveness. Implementation of therapeutic drug monitoring will potentially require additional hospital attendance by patients to measure trough drug concentrations (for self-injected medications), laboratory set-up and standardization and an interpretation service, in addition to the financial costs of the assays. Although therapeutic drug monitoring has become the preferred practice for

the treatment of inflammatory bowel disease in the USA, there is a dearth of evidence as to whether therapeutic drug monitoring improves clinical outcomes and, particularly, whether this approach can be cost effective[125]. Consequently, the UK National Institute for Health and Care Excellence does not recommend routine therapeutic drug monitoring in patients with either RA or Crohn's disease but does recommend further research in this area[126]. By contrast, although limited evidence was available, a systematic review of studies in patients with inflammatory bowel disease suggests that this approach has cost-saving benefits (particularly for reactive therapeutic drug monitoring), as well as potential benefits in terms of improving TNF inhibitor durability (particularly for proactive therapeutic drug monitoring)[127]. Importantly, if therapeutic drug monitoring does become a cost-effective addition to the care of patients receiving biologic agents in rheumatology, education for health-care professionals and patients would be required concerning the different types of assay platforms available, their standardization and their interpretation.

Conclusions

The advent of biosimilars and the need for rigorous regulatory standards have catalysed research and innovation in the measurement of immunogenicity, resulting in a better understanding of its determinants, consequences and clinical implications. Furthermore, a variety of methods now exist for characterizing ADAs, which have highlighted differences in immunogenicity among different biologic agents. Although the measurement of circulating biologic drug concentrations in concert with ADA measurements can, in theory, optimize dosing strategies, the attractiveness of therapeutic drug monitoring is not yet supported by high-quality cost-effectiveness studies, which will be required before such testing becomes a part of standard care. Additionally, the simple methods for therapeutic drug monitoring that have appeared in the literature should not detract from the sophistication of the assays used, which demand a degree of interpretation by the requesting clinicians, as well as education of the patients themselves.

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