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# How the immune system responds to therapeutic biological agents

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**Abstract**

Biological agents target disease mechanisms and have modified the natural history of several immune-mediated disorders. Biological agents are structurally immunogenic, and therefore usually elicit a minor, subclinical and transient phenomenon. Occasionally, however, these drugs induce complete cellular and humoral immune responses, with the main clinical consequences being hypersensitivity reactions or loss of treatment response. This article considers the relative pathogenic mechanisms influencing immunogenicity in biological agents and discusses mechanisms of tolerance and adaptive immune response, including adaptive T-regulatory cell induction and immune response induction. Methods of determining cellular and humoral immune response to biological agents are identified and examined. Assays to detect antidrug antibodies and their isotypes can assist in monitoring immunogenicity and in preventing adverse events. Such strategies also enable resource conservation and may provide regulatory authorities with new insights that can be useful during the process of approving new biological or biosimilar agents.

**Keywords**

Adaptive response, antidrug antibodies, biologicals, humoral response, immunogenicity, immune response, tolerance

## Introduction

In the last decade, several treatments have been developed to target disease mechanisms. These biological agents have modified the natural history of immune-mediated disorders such as rheumatic diseases, inflammatory bowel diseases, systemic vasculitis and psoriasis.[1–3] These treatments are

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structurally immunogenic and usually elicit a minor, subclinical, transient phenomenon.[4] However, they may occasionally induce complete cellular and humoral immune responses,[5] the main clinical consequences of which are drug hypersensitivity and loss of treatment response.[6] The study of immunogenicity in therapeutic biological agents is an important research tool, particularly considering the availability of biosimilars (i.e., copies of original biological agents that are manufactured by a different company, once the patent on the original product has expired).

## Factors influencing immunogenicity

Several factors may contribute to developing an immune response to biological agents. Generally, the expression of specific human leukocyte antigen (HLA) haplotypes might contribute more readily to antidrug antibody development in subjects who recognise the wild-type protein as a foreign epitope, even if specific data are not yet available. It has also been shown that the high expression of costimulatory molecules on dendritic cells may accelerate the outgrowth of antidrug antibodies.[7]

It is well known that high doses of biological agents foster tolerance mechanisms and reduce their immunogenic activity.[8] An inverse correlation between drug dose and antidrug antibody level has been reported in patients with rheumatoid arthritis or Crohn's disease.[9,10] Drug tolerance can arise during long-term treatment regimens with intravenous, rather than intramuscular or subcutaneous, treatment administration.[11] Immunogenicity is also reduced when biological agents are administered in combination with traditional immune suppressors.[12,13]

Despite showing low xenoantigen sequences, all monoclonal antibodies (mAbs) display new, potentially immunogenic epitopes. For example, fully human mAbs (such as adalimumab), which lack foreign epitopes, may also elicit antidrug antibodies due to differences in glycosylation or to the sequences of the mAb idiotype.[14] $F_c$ fusion proteins include few new epitopes, however, thus explaining the low degree of immunogenicity with etanercept and abatacept.[15]

Antiadalimumab antibodies always have a neutralizing effect, whereas different percentages of neutralizing antidrug antibodies have been reported for other tumour necrosis factor (TNF)-$\alpha$ blockers.[5,16] Notably, antidrug antibodies to etanercept never have a neutralizing effect.[17]

## Mechanisms of tolerance and adaptive immune response

The immune response to self proteins is controlled by mechanisms shared by biological agents. The mechanisms of adaptive T-regulatory (Treg) cell induction are not well known, and may include both regulatory cytokines and intracellular signaling (cytotoxic T-lymphocyte-associated protein [CTLA-4], programmed cell death protein 1 [PD1], etc.).[18] Adaptive Treg induction is associated with sustained tolerance and probably requires the concomitant presence of Treg cells with the same specificity as the self-reactive T cells.[18]

High-dose tolerance, involving anergy and immune deletion, is likely responsible for the nonresponsiveness of T cells to therapeutic biological agents. It has been shown that some sequences located in the $F_c$ and $F_{ab}$ domains of human immunoglobulin (Ig) G exert a central role in immune tolerance. These epitopes (known as Tregitopes) selectively expand Treg cells but not T-effector cells.[19]

Other regulatory mechanisms include the intrinsic activity of biological agents. Infliximab and adalimumab, for instance, act as a reverse signal on membrane

TNF-α-bearing cells by inducing tolerogenic dendritic cells and upregulation of signals (Notch 1), thus mediating inhibition of the T-cell cycle.[20,21] The induction of immune responses to biological agents probably occurs by two main mechanisms: (i) activation of an adaptive immune response to non-self epitopes on the drug; and (ii) the loss of immune tolerance. Most humoral responses to biological agents are due to an adaptive response to foreign antigens, leading to the expansion of memory T (and adaptive Treg) cells, and B cells specific to foreign epitopes.[5]

The sequence of events leading to B-cell activation and antidrug antibody production can follow a T-independent or a T-dependent process. The former occurs when some structural sequence of the drug (polymeric repeats or protein aggregates) induces the signals required to directly stimulate B-cell subsets, and usually does not lead to affinity maturation or generation of memory B cells. In contrast, T-dependent B-cell activation results in a more robust antibody response, isotype switching and induction of memory B cells.[22] The prevalent induction of high affinity antidrug antibodies of the IgG class or IgG1/IgG4 subclasses reinforces the concept that biological agents predominantly act as T-dependent antigens.[23] Naturally, this mechanism also requires T-cell recognition of immunodominant epitopes in the context of HLA Class I/II molecules of antigen presenting cells, and the amplification of central-memory and effector-memory T cells.[24] Therefore, T cell recognition of drug peptides is a prerequisite for generating memory B cells and for antidrug antibody formation.[25]

## Assessment of cellular and humoral immune response

Detection of memory T cells specific for drug epitopes includes proliferation assays and cytokine production by freshly isolated mononuclear cells or T-cell lines expanded in vitro with the drug (or its peptides). A second approach to identifying T-cell epitopes is an in silico method, which enables the prediction of the binding affinity of peptides along the entire sequence of biological agents to HLA class I or II antigens.[26] A third method is studies of genetic linkage between HLA haplotypes and antidrug antibody outgrowth. [26]

Different methods have been reported for the assessment of humoral response to biological agents including double antigen (bridging) enzyme-linked immunosorbent assay (ELISA), sandwich ELISA, radio immunoassay (RIA), surface enhanced laser desorption/ionization mass spectrometry and surface plasmon resonance.[27] Comparison of different studies is at present difficult since the assays are not standardized or validated. This raises some concerns regarding the validity of certain studies on this topic.

Bridging ELISA for the detection of antidrug antibodies is influenced by circulating drugs and Ig with rheumatoid factor activity.[28] New immunoassay approaches, such as acid dissociation bridging ELISA, may increase the sensitivity of antidrug antibody detection.[29]

The presence of biological agent-specific IgE can be detected using the ImmunoCAP® platform (ImmunoDiagnostics, ThermoFisher Scientific, Waltham, MA, USA) or RIA. The identification of mAbs-specific IgE may be difficult because of the small quantities of this isotype in human serum and interference from IgG.

When a hypersensitivity response towards a biological agent is suspected, skin tests must be performed. Positive skin tests are suggestive of an IgE-mediated mechanism, as demonstrated in mAbs-reactive patients.[28,30]

## Conclusions

Advances have been made in the knowledge of pathogenic mechanisms related to

immunogenicity of biological agents and their consequences. The use of new assays to detect antidrug antibodies and their isotypes may be useful for monitoring immunogenicity and preventing adverse events. Such a strategy also enables us to save resources and may provide the regulatory authorities with new insights, which could be useful during the process of approving new biologicals or biosimilars.

## Declaration of conflicting interest

The authors declare that there are no conflict of interest.

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