

Self or Non-Self, that is the Question

Sandra Hageman and Edwin Janssen at Eurofins Medinet investigate immunogenicity and the factors that influence the development of unwanted immune responses

For the past two centuries, scientists have made a great effort to unravel the essence of the human immune system – how our bodies distinguish between self and non-self. Many credit Edward Jenner for his work to induce immunity for smallpox disease. In the late 18th century, smallpox was severe and life-threatening; one in three who contracted the disease would die, and those who survived were often badly disfigured. Milkmaids however did not generally get smallpox, and Jenner and many of his contemporary scientists explored the possibility that the pus in the blisters which milkmaids received from cowpox protected the girls from smallpox. The vaccination campaigns that originated from this research eventually led to the declared eradication of smallpox in 1980 (1,2).

A thorough understanding of how our immune system works has now been gained. In humans, immunity against foreign substances or microorganisms that invade the body is divided into two major categories: innate immunity and acquired immunity. Innate (natural) immunity is present from birth and consists of many factors that are relatively non-specific, such as the protection from the skin barrier. Acquired immunity is a more specialised form of immunity and is a consequence of an encounter with a foreign substance. There are three major cell types that participate in acquired immunity:

- ◆ The first consists of B lymphocytes, which synthesise and secrete antibodies specific to the foreign substance (humoral immune response)
- ◆ The second is made up of T lymphocytes, which produce cytokines to activate macrophages, natural killer cells, and antigen specific cytotoxic T cells (cellular immune response)
- ◆ The third cell type consists of macrophages – phagocytic cells which do not exhibit specificity against the foreign substance

Macrophages are involved in the processing and presentation of foreign substances to T lymphocytes (antigen presenting cells). Under some circumstances, the immune response, rather than providing protection, produces damaging and sometimes fatal results. Such reactions are collectively known as hypersensitivity reactions. It should

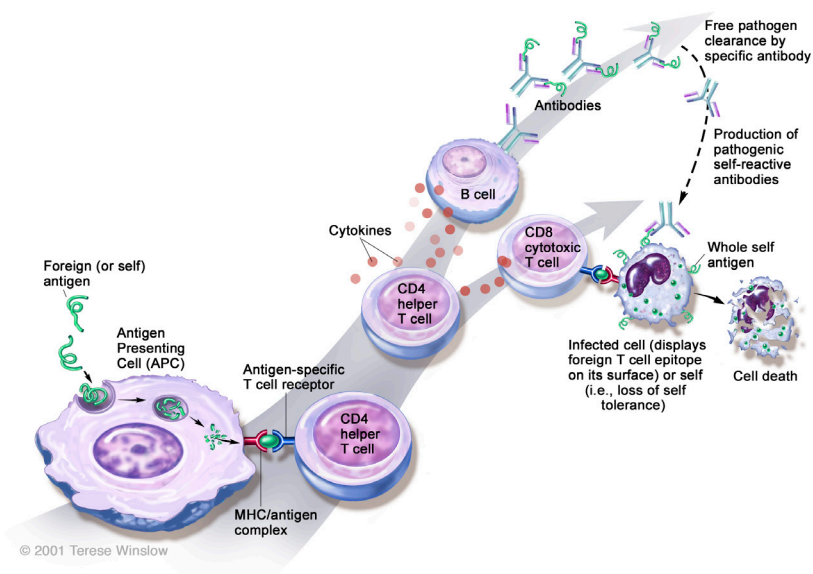
be remembered that they differ from protective immune reactions only in that they are exaggerated or inappropriate, and ultimately damaging to the host (1).

IMMUNOGENICITY OF BIOPHARMACEUTICALS

As shown in Figure 1, our immune system builds up a natural defence that has evolved over time, leading to tolerance or immunity. Within the pharmaceutical industry, biopharmaceuticals have become a very important area of research. For more than 20 years, biopharmaceutical drugs have been increasingly used in the marketplace to complement more traditional small molecule drugs, and they have revolutionised the treatment of many diseases. Biopharmaceuticals consist mainly of drug products containing biotechnology-derived proteins as the active substance. These biotechnology-derived therapeutic proteins have been observed to provoke immune responses in the human body, to varying extents. The possibility that, or the degree to which, a particular substance may provoke an immune response is described as its ‘immunogenicity’. The immunogenicity of a biopharmaceutical is a function of its ‘foreignness’ to the recipient, a certain minimal molecular weight and a certain degree of physicochemical complexity (1).

Immunogenicity can be either wanted or unwanted. Biopharmaceuticals provoke a wanted immune response when the immune response is directed against substances administered for the induction of a protective immunity, such as in the smallpox example describe above. Furthermore, in

Figure 1: Immune response to self or foreign antigens



some cases, biopharmaceuticals should specifically stimulate targeted immune responses through activating antibody dependent cell-mediated cytotoxicity or complement-induced cell death, such as the use of the CD20-specific antibody rituximab for the treatment of CD20+ follicular lymphoma (2).

Unfortunately, unwanted immune responses have also been observed, and the consequences of an unwanted immune response to a therapeutic protein may range from transient appearance of antidrug antibodies (ADA) without any clinical significance, to severe life-threatening adverse reactions.

The ADA may negatively influence the therapeutic effect of the biopharmaceutical, resulting in a need to enhance the administered dose (PK effect) or, more dramatically, resulting in a negative effect on drug efficacy. Furthermore, clinical safety issues may occur, such as anaphylactic shock, non-acute hypersensitivity or cross reactivity with the endogenous counterpart, causing deficiency syndromes in the patient. Needless to say, immunogenicity may have potential implications for both the safety and efficacy of biotechnology-derived therapeutic proteins (3,4).

BIOSIMILARS AND IMMUNOGENICITY

The production and manufacture of biopharmaceuticals usually occurs in living cells. The product and host-related factors therefore have the potential to provoke an immune response. With the patent expiry of many first-generation biopharmaceuticals, the development of biosimilars (generic products) becomes highly attractive. However, the development of biosimilars may result in differences in product composition, manufacturing and packaging from the original molecule, and therefore may induce unwanted immunogenicity, similar or different to the original compound.

A well-known example of the immunogenic potential of a biopharmaceutical drug illustrates this point. In 2002, Janssen-Ortho, now Johnson & Johnson, had been under investigation by health authorities with regard to reported post-marketing adverse reactions to their recombinant human erythropoietin Eprex (r-HuEPO). The drug consists of *epoetin alfa*, similar to the human erythropoietin (EPO) hormone produced in kidneys which stimulates the production of red blood cells. Eprex belongs to a class called erythropoiesis-stimulating agents (ESA), and is used to increase the levels of red blood cells in patients with chronic renal failure (CRF) or who are suffering from anaemia caused by chemotherapy. There was a sudden rise in patients developing a rare condition called pure red cell aplasia (PRCA), and most of those patients had administered the drug subcutaneously. Apparently, via this specific administration route, the drug had been recognised as a non-self protein, and antidrug antibodies were attacking both the engineered EPO as well as the patient's own EPO, resulting in a potentially life-threatening condition. In addition, the peak of PRCA cases overlapped with changes to the formulation of the biopharmaceutical drug (5). In spite of these rare induced adverse reactions, the ESA class of biopharmaceutical products – including Eprex – raised \$10 billion in 2008, ranked

at fourth in the Top 20 Biologics 2008, proving that patients have a lot to gain from taking these type of drugs (6).

Thus, it is of pivotal importance to monitor adverse events closely when treating patients with biotechnology-derived therapeutic proteins and biosimilars. An unwanted immune response may be provoked by their intrinsic characteristics, as well as the manufacturing and packaging process, where slight changes in manufacturing conditions could result in the onset of immunogenicity.

EXTENSION OF IN-HOUSE SCIENTIFIC PHARMA TEAMS

With the strong progression of the complexity of biopharmaceuticals being developed, the need for outsourcing the development of speciality assays has become apparent as well, including immunogenicity testing throughout the clinical development process. Although the potential risks of immunogenicity of a biopharmaceutical can be predicted by performing *in vitro* tests and *in vivo* animal studies during drug development, the immunogenic potential, and thus safety, of a biopharmaceutical drug can only be assessed through clinical trials (7).

IMMUNOGENICITY TESTING IN CLINICAL TRIALS

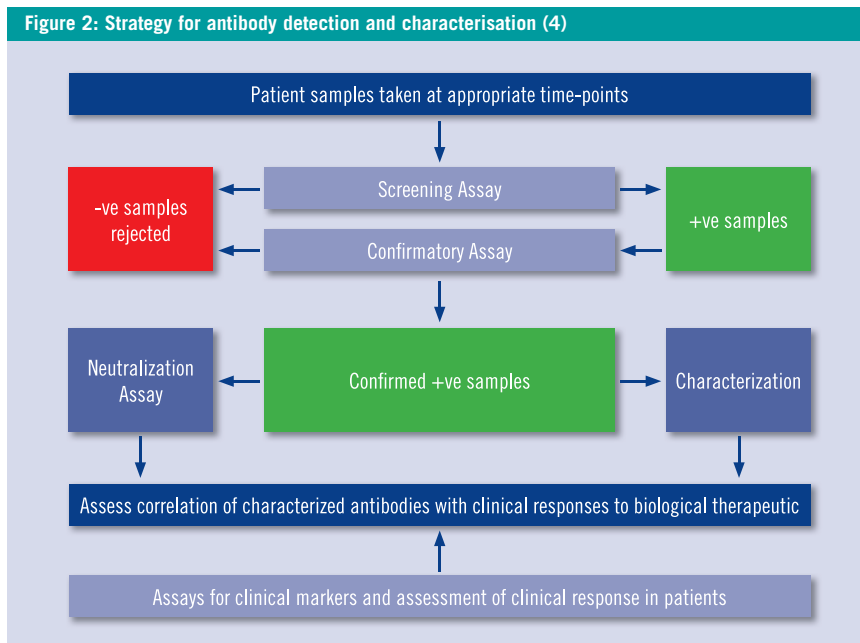
The detection and characterisation of wanted and unwanted immune responses towards new biopharmaceuticals is essential for ensuring their clinical safety and efficacy. The European Medicines Agency (EMA) recently released guidelines which include recommendations to incorporate immunogenicity testing as part of all clinical trials evaluating all patients, as the unpredictability of the onset and incidence of immunogenicity requires long-term monitoring (4).

For vaccine-mediated wanted immune responses or stimulation of wanted immune responses, positive patient samples have been screened and identified in all phases of clinical development. In addition, an inhibition enzyme-linked immunosorbent assay (ELISA) can be used to determine the IC50 of vaccine-mediated immunity.

In the majority of cases, the assessment of drug-induced unwanted immune response consists of screening for a humoral response. A comprehensive step-by-step workflow is followed to support immunogenicity testing in all phases of clinical trials (Phases I to IV). The first step is to select and develop tailor-made ELISA-type assays to detect and characterise antidrug antibodies that may be present in the patient's blood sample. It is of pivotal importance to screen person by person, as considerable inter-individual variability in antibody response with regard to antibody class, affinity and specificity has been observed.

After this initial screening round for the identification of antibody positive specimens, positives are then confirmed using a competition assay with the biopharmaceutical drug of interest, confirming the specificity of the observed response. These assays can also determine immunoglobulin class

Figure 2: Strategy for antibody detection and characterisation (4)



(isotype-specific) responses, which can elucidate if the detected immune response is the primary (IgM) or the secondary (IgG) response to the immunogenic stimulus. When ADA are produced that bind to the biopharmaceutical compound *in vitro*, it does not necessarily mean that they will also inhibit the therapeutic effect *in vivo*. The next step comprises an assessment of the neutralising capacity of the ADA formed, using either functional bioassays or a competitive ligand binding ELISA.

About the authors



Sandra Hageman graduated in biochemistry and extended her education with postgraduate business school. After working at the Netherlands Cancer Institute in Amsterdam for three years, she started to build a track record in business development and marketing in the life science industry. Sandra joined Eurofins Medinet in 2006 as Product Manager, and has gained experience in global marketing and communication for the global central laboratory services of the organisation.
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In some cases, the drug-induced unwanted immune response consists of a cellular response. These T lymphocyte mediated immune responses induce cytokines to activate macrophages, natural killer cells, and antigen-specific cytotoxic T cells, and do not produce ADA. Therefore, the screening for humoral response as described above is not applicable. Instead, T lymphocyte cell surface markers, intracellular cytokine levels and released cytokines are determined through FACS and T cell ELISPOT analyses.

CONCLUSION

Unwanted immunogenicity may have potential implications for both the safety and efficacy of biotechnology derived therapeutic proteins. Regulatory bodies recommend monitoring immunogenicity as part of all clinical trials and evaluating all patients, as considerable inter-individual variability in antibody response with regard to antibody classes, affinity and specificity has been observed. In addition, the unpredictability of the onset and incidence of immunogenicity requires long-term monitoring, both during clinical trials and in post-marketing surveillance. Biopharmaceutical companies can team up with a global central laboratory services supplier who can take the specific immunogenicity assay tailored to the individual biopharmaceutical drug from early development to post surveillance. In this way, safety assessment is combined with immunogenicity testing in a synergetic approach.

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