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Editorial

Understanding the Immunogenicity Concept☆

Comprender el concepto de inmunogenicidad

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Immunogenicity is just one of the many benefits of our immune system and is defined as the ability of different substances to trigger an adaptive cellular and humoral cell immune response that is long-term and leads to immunological memory.

In rheumatology and other medical specialties such as dermatology, gastroenterology, neurology and immunology, biological proteins derived from biotechnology are increasingly used as therapeutic agents. These proteins, like many other exogenous agents can induce humoral and cellular immune responses. However, in recent years we have noticed how these concepts have been interpreted haphazardly, and sometimes in the wrong way from the point of view of their clinical significance.

The immune response or immunogenicity which can be triggered by a therapeutic protein has been proposed as a cause of potential clinical events, including hypersensitivity reactions, decreased effectiveness of the therapeutic molecule and induction of immune processes, including the formation of antibodies against the protein in question.[1,2] The most dreaded immunogenicity in clinical terms occurs due to the so-called neutralizing anti-drug antibodies which, as their name implies, have the ability to bind the agent (drug) and neutralize it, preventing its biological or therapeutic function.[3]

Many factors can influence the immunogenicity of therapeutic proteins, including those inherent to the patient, the disease itself or those related to the product itself. The determinants of patient-related immunogenicity that may predispose unwanted immune responses include the underlying disease, genetic factors and baseline immune status, including associated immunomodulatory therapy. Furthermore, product related factors also influence the probability of inducing an immune response; for example, the route of administration, the type of protein, aspects of the manufacturing process, impurity profile or excipients and formulation characteristics, stability, degradation products or aggregates, dosage, the dosing interval and treatment duration.[4–6]

The immune response mechanism directed against therapeutic biological molecules seems to be due to "transient" loss of immunologic tolerance, rather than the classic immune response to an exogenous protein, because when these agents are suspended, antibodies may even disappear.[6,7] Some patients may develop antibodies which neutralize the biological activity of the drug, while others may develop antibodies that bind to the product, altering the pharmacokinetics or pharmacodynamics, compromising its effectiveness in both situations, but with different stages.[8–13]

The intrinsic immunogenicity of a drug varies considerably between biological molecules. For example, interferon-γ (IFN-γ) and granulocyte colony stimulating factor may be capable of inducing an immune response that will eventually compromise their effectiveness, but appropriate techniques are yet to be developed for detecting them. In addition, molecules such as colony stimulating factor, granulocyte–macrophage, IFN-α and IFN-β appear to be inherently immunogenic.[12,14–16]

Data on possible immune responses to therapeutic proteins must be determined and always adduced before the drugs release, but of course there are events that may occur in the post-marketing period of the drug. When undergoing application for marketing authorization, the manufacturer of a drug derived from biotechnology should include a summary of research regarding its immunogenicity.

The EMEA (*European Medicines Agency*)[17] guide recommends that the evaluation of immunogenicity be performed using validated tests for antibody determination and characterization of the immune response observed regarding safety and efficacy, establishing a correlation between the presence of antibodies and its pharmacokinetics and antibodies. Furthermore, it is recommended that the role of immunogenicity be evaluated in certain events, such as hypersensitivity, infusion reactions, autoimmunity and loss of efficacy, providing at the same time, information relevant to the adverse event. While the guide briefly describes the different methods that can be used to evaluate the immunogenicity, it does not specify any particular methodology.

How Are the Therapeutic Antiprotein Antibodies Developed (Commonly Known as Anti-Drug Antibodies)?

An antibody is a particular type of protein produced by an immune system cell, the B lymphocyte. Each antibody molecule has two interconnected different amino acids polymers, one of these known as the heavy chain and the other as the light chain. An

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antibody has two heavy chains and two light chains, each heavy chain is about 450 amino acids long and each light chain has 250 amino acids.[18]

Each B cell produces, when faced with certain stimuli (antigens, in this case immunogenic therapeutic proteins), antibodies that differ from those of antibodies produced by other B cells. The difference is subtle, but critical in recognizing and fixing antigen. The antibody site (paratope) that binds to the epitope of the antigen that triggered its formation is very specific. The paratope "recognizes" a particular molecular shape and if the shapes are not complementary, then it does not bind to the molecule. Thus, the antibody, although formed under the stimulus of a particular antigen, neutralizing or not is not effective against this antigen it has induced.[18,19]

The antibodies produced by B lymphocytes, specifically by plasma cells, which are more differentiated forms of this cell line are found in the peripheral blood and in the lymph. The antibodies generated in response to a foreign substance with which we have somehow come into contact, such as a bacteria, viruses (after vaccination or infection) or therapeutic proteins, may be found anywhere on the body. In addition, when these circulating antibodies come in contact with the antigen against which they were generated, they bind to it. This union will have several possible outcomes-inactivatation of the "antigen", so it can be more easily destroyed by macrophages (cells that engulf the antigen coated with antibody molecules) or covering of the binding sites of the "antigen" to the target cell and preventing its entrance. If contact was reestablished with the antigen, the immune response would lead to an improvement in producing more specific antibodies, without hardly perceiving it. This phenomenon, known as immunological memory, which in some cases is beneficial to the body, may be responsible for the failure of different protein-based therapies which are very useful under certain conditions.[18–20]

Have We Reached the Point of Being Able to Unify Criteria for the Detection of Neutralizing Anti-Drug Antibodies?

The standardization of routine laboratory procedures to detect neutralizing anti-drug antibodies, remains a huge challenge. Controversy due to the variability of the results obtained in different populations in studies carried out so far does not allow us to unify criteria and establish objective parameters to determine the presence of anti-drug antibodies used for the treatment of various diseases. Furthermore, the presence of anti-drug antibodies is not always a risk factor for the patient in terms of side effects, or something that predicts primary or secondary efficacy loss.

In connection with the standardization of the techniques for the detection of antibodies, we have a clear example in the field of rheumatology. Throughout the nineties there was discussion on the procedures used for the detection of antibodies to cyclic citrullinated-peptide in view of the high variability of the results obtained and published.[21–24] But today no one doubts the positivity or negativity of the result of anti-CCP reported by a routine laboratory in diagnosing and treating a patient with rheumatoid arthritis, as it has been shown, through standardization, high specificity and sensitivity, not only a highly predictive, intra- and interassay reproducibility, but also intra- and interlaboratory agreement, making the test cost effective and providing a key tool for the diagnosis, prognosis and treatment implementation of early onset RA.

In this sense, a prerequisite for immunogenicity studies is associated with the detection and characterization of antibodies using methods that are both reliable and, above all, reproducible.[25,26] Thorpe and Wadhwa[13] have reviewed several techniques currently available to determine the presence of antibodies generated against therapeutic proteins in biological fluids; for example, immunoassays (Enzyme-Linked Immuno-Sorbent Assay, ELISA), radioimmunoprecipitation (RIA), useful as screening procedures, "surface plasmon resonance", expensive, automated procedures that demonstrate antibody–antigen interactions in real time and, above all, bioassays, which are essential to determine whether or not antibodies are neutralizing.[13,25,27] Each of these methods has advantages and disadvantages, and it is noteworthy that neither alone is sufficient to define the characteristics of the antibodies that are produced against a biological molecule. Applying a strategy involving the use of various methods is necessary for a thorough understanding of the amount and the type of antibodies generated against a therapeutic product.

Current Controversy Regarding Anti-Drug Antibodies

In a review published in 2008, Purcell and Lockey[28] describe the term immunogenicity applied to numerous therapeutic agents, including coagulation factors, growth factors, hormones, enzymes, cytokines, monoclonal antibodies including recombinant insulin, an identical human insulin which is less immunogenicity, logically, than porcine or bovine derived insulin.[29–31] We have not achieved, however and despite numerous studies, the identification of the clinical significance of antibodies to recombinant insulin.[32]

Many factors may contribute to the alteration in the structure of the protein to elicit an immune response including antibody formation, glycosylation, pollutants; changes in temperature and the storage media may also have a decisive role.[3,33,34] Such is the case of a formulation of IFN-α, which is determined to be abnormally oxidized at room temperature, changing the tertiary structure of the protein leading to an immune response with antibody production. Later, a change in storage and formulation resulted in a reduction of the formation of substantial anti-drug antibodies.[15]

The clinical consequences of anti-drug antibody formation are extremely variable; while some have no clinically relevant effect (recombinant insulin antibodies), others have side effects ranging from loss of efficiency to effects that may even endanger the life of the patient, as is the case of pure red cell aplasia induced by recombinant erythropoietin, an effect produced by an IgE-independent anaphylaxis.[34,35]

Also noteworthy are the recombinant factor VIII and factor IX which were developed in the late eighties for the treatment of patients with hemophilia A and B, respectively. Although the use of these therapeutic agents was successful in the treatment of these diseases, the formation of neutralizing antibodies was a significant problem directly related to the severity of the disease. The incidence of antibody formation to recombinant factor VIII was 15%–35% in mild-moderate forms of hemophilia A, and in severe cases, where F VIII produced naturally was practically absent (<5%), antibody formation was 52%.[36,37]

In rheumatology specifically, the lack of response, loss of efficacy or reactions to treatment in connection with the use of monoclonal antibodies against TNF-α or its soluble receptor have been directly related to the development of anti-drug antibodies (anti-human chimeric antibody, HACA) and low or undetectable plasma levels of the same.[38,39] While the concept of anti-drug neutralizing antibody is not demonstrated *in vivo* for these therapies the reality is that a percentage of patients develop these antibodies in association to the loss of response or infusion reactions.

Some studies report a prevalence of HACA by ELISA in rheumatoid arthritis patients ranging from 17.45% to 44% for anti-TNF-α and in a range of 15% to 30% in patients with ankylosing spondylitis,[40–43]. Different statistics are seen when using a different technique of ELISA detection.[44,45] It has been speculated that the presence of HACA may explain cases of treatment failure, but not

all nonresponders have HACA, suggesting that other factors may be involved in treatment failure.

The immunogenicity of anti-TNF is dependent on its structure, being higher when using those of murine origin compared to the humanized or those modified by genetic recombination.[46] In the latter, it would be virtually impossible to detect the paratope binding the antigen epitope and, therefore, difficult to develop ELISA to quantify it. Heterogeneity in the techniques for detection (ELISA, RIA) and nonstandard procedures make it impossible to know the real meaning of the presence of these antibodies. Discrepancies between the presence of anti-drug antibodies and low drug levels and side effects point increasingly to the levels of drug than to the development of antibody itself, as the main cause of loss of effectiveness.[47,48] Furthermore, inconsistent results regarding different anti-TNF in various diseases do not explain the variations between the presence of anti-drug antibodies and the loss of response associated with each disease and every therapy.[40,41,48–50]

While there appears to be a statistically significant association between the presence of anti-drug antibody-levels and drug levels and efficacy loss-infusional reaction according to data from different research groups using different techniques, we do not know in detail the clinical application (positive predictive value, negative predictive value, odds ratio or relative risk), nor the intra-interassay reproducibility according to the procedures followed, and intra-interlaboratory variations according to the techniques used that these findings may offer for a variety of diseases, for different patients and with different therapeutic strategies.

Conclusions

A lot of factors may be responsible for variations in the profile of immunogenicity of different biological products, which makes for cautious interpretation of this data. The results could be truly comparative if the molecules were evaluated under the same conditions, using the same clinical and laboratory protocols, and determining the anti-drug antibodies using standardized procedures in all laboratories.

No doubt immunogenicity plays an important role in the therapeutic effect of a drug, but previous examples, such as insulin or recombinant erythropoietin, make us cautious in interpreting the results for those drugs commonly used in our specialty. Other factors to consider, such as the route of administration or drug conservation, the clinical profile of the patient and the type of illness or changes in the detection methods need to be weighed and analyzed before the clinical relevance of this phenomenon is determined.

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