Immunogenicity of Biological Agents: Basic Knowledge and Clinical Implications

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Abstract - Immunogenicity of biological agents leads to the development of anti-drug antibodies (ADA) and it may be associated to all biologicals irrespective of the presence of xenoantigens. Several drug-, patients-, disease- and treatment-related factors contribute to immunogenicity. Low ADA levels can influence the efficacy of the drugs, whereas high levels can induce hypersensitivity reactions. Immediate infusion reactions occur during or within one hour after infusion, and their clinical manifestations range from mild to severe. The application of novel methods for detecting ADA has allowed to distinguish between the involvement of IgE- or non-IgE isotypes. It is important for clinicians to recognize symptoms of reactions, but also their pathophysiological mechanisms to evaluate risk assessment and prophylactic regimens. The studies on immunogenicity may help to determine optimal treatment regimen required to minimize the likelihood of ADA onset and related side effects. This review summarizes i) the epidemiological data on immunogenicity of biologicals leading to the loss of response to the treatment or to hypersensitivity reactions, ii) the current knowledge of factors influencing immunogenicity to biologicals as well as the pathogenic mechanisms of drug-induced hypersensitivity reactions, iii) the assays to monitor immunogenicity and iv) the risk factors to prevent hypersensitivity reactions.

Keywords – Anti-drug antibodies, Biological Agents, Immunogenicity

I. INTRODUCTION

During the last decade new treatments targeting the disease mechanisms, referred as biological modifier therapies, are revolutionizing the natural history of several immune-mediated inflammatory disorders and malignancies. Many biological agents (BA) are now available as new therapeutic principles, including recombinant cytokines, monoclonal antibodies (mAbs) and fusion proteins and many others are in progress to be clinically approved by regulatory authorities. BA are structurally immunogenic and their chronic administration is able to elicit some response from the immune system. Most often it is a minor, subclinical and transient phenomenon, but, sometimes, these drugs induce an adaptive immune response which may impact the efficacy of drug with loss of response (LOR) and, in some cases, elicit the outgrowth of hypersensitivity reactions (HR). Thus, the safety of BA, and particularly of therapeutical mAbs, represents an important tool of research by considering their growing number (in particular of the biosimilars) and expanding clinical applications.

Epidemiological data: correlation between immunogenicity and LOR or HR

The increasing use of BA in the treatment of immuno-mediated disorders and cancer paralleled the data on the outgrowth of immunoreactivity to the drugs. Indeed, in some patients the stimulation of immune response results in the onset of anti-drug antibodies (ADA). The ADA detection has been correlated with the unresponsiveness to treatment initially to TNF-α blockers (infliximab and adalimumab) and then to other mAbs as natalizumab (anti-α4 integrin mAb) or recombinant clotting factors and type I interferons.

The prevalence of ADA to infliximab has been reported to range between 10 and 60% of patients [1-5]. This large range can be explained by differences among groups of patients, of concurrent medications, timing of sampling, duration of follow up, drug dosing as well as the assay to detect ADA. In Rheumatoid Arthritis (RA) patients, adalimumab, a fully anti-TNF-α-specific human antibody, induced ADA in 5% of patients and the concomitant methotrexate treatment

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resulted clearly protective [6-8]. In patients with Multiple Sclerosis, the treatment with natalizumab induced ADA in about 9% of subjects [9] and is associated with reduced efficacy and infusion reactions [10]. A post-marketing surveillance indicated that low titres of ADA are detectable in a few number of subjects treated with omalizumab (a humanized mAb recognizing the ε3 domain of human IgE) without clinical associations [11]. IgG antibodies against this drug were transiently detected when it was administered in the inhaled form [12].

Since the large use of TNF-α blockers in the last decade, epidemiological data on LOR and HS are available. Overall, the frequency of infusion reactions to infliximab is low, approximately 5% of treated patients [13, 14]. Clinical data from more than 1600 RA patients indicated that infusion reactions occurred in only 4.8% [15]. Acute infusion reactions were reported to occur in 3.8% of infliximab-treated patients with Crohn’s disease [16]. In patients with psoriatic arthritis, only 1% of subjects developed a severe infliximab infusion reaction. These proportions are lower than those observed in other clinical trials (22%) and in previous uncontrolled series (5-19%) [17-21]. In a heterogeneous cohort of more than 100 patients we observed a very low frequency of infusion reactions (1.5%), a higher proportion of reactions being in RA patients (3.4%) compared to other diseases [22]. Type I infusion reactions occurred most commonly during the first (3-6) infusions [23]. Moreover, in these patients we showed a tight relationship between the onset of ADA and the acute reaction [24]. The high concentration of ADA correlated with the precociousness, frequency and severity of infusion reactions compared to those of patients with low titre of ADA [23,24].

The proportion of immuno- and non-immuno-mediated reactions to infliximab is lower than that of other chimeric mAbs, such as cetuximab (anti-Epidermal Growth Factor receptor mAb) and rituximab (anti-CD20 mAb) [13, 14]. Mild to moderate infusion reactions were usually associated with the first infusion of cetuximab, the incidence ranging from 12% to 19% [25-27]. Among other mAbs used in oncology, rituximab and trastuzumab are the most frequent cause of acute infusion reactions (10-15%) [28, 29].

Among humanized mAbs, natalizumab causes severe reactions in up to 1% and mild-to-moderate reactions in about 4% of patients [10, 30]. By contrast, omalizumab used in severe allergic asthma, is usually well tolerated and it has been found to induce anaphylaxis in approximately only 0.1–0.2% of patients [31-33].

Factors influencing Immunogenicity

MAbs are generally well tolerated in humans, despite they contain sequences that may be recognized by the recipient as non-self epitopes, stimulate immunity, and lead to the production of specific ADA [34]. As expected, the recognized epitopes mainly include xenoantigens present in the murine part (anti-mouse antibodies) of mAbs. Molecular technology has enabled the structure of mAbs to be fine-tuned for specific therapeutic actions and to minimize immunogenicity [35]. Engineered therapeutic mAbs are of the IgG isotype and classified as: i) chimeric monoclonal antibody, made from variable regions of a murine source and constant regions of a human immunoglobulin; ii) humanized monoclonal antibody, containing only the complementarity-determining regions (CDR) of a murine immunoglobulin with the remaining part from a human source; iii) human mAbs, completely of human origin.

The differences of amino-acid sequences related to the mouse origin of mAbs are also relevant for immunogenicity. Even though the techniques for producing these reagents evolved in the last years with less xenogenetic sequences, they display new T and B cell epitopes which are potentially immunogenic. Also fully human mAbs produced by using phage and humanized mouse models, which virtually lack foreign epitopes, may elicit ADA, due to differences of glycosilation or to the intrinsic immunogenic sequences of mAb idiotype. The Fc fusion proteins express reduced numbers of new epitopes compared to full mAbs (virtually only in the linker region), thus explaining the low degree of immunogenicity of such biologicals as etanercept or abatacept [36]. Lastly, the absence of Fc fragment (as in certulizumab) decreases not only the cytolytic events due to Fc binding, but also the uptake/processing by APC of drug-target immunocomplexes, thus impairing immunogenicity [37].

Among drug-related factors the target-binding ability is relevant for immunogenicity. The treatment with a modified alemtuzumab lacking a single amino-acid in the binding site of the molecule, induced a lower ADA production compared to patients treated with the therapeutic form of the drug [38]. Also the size of immunocomplexes between the drug and the target molecule condition immunogenicity. For instance, etanercept forms small immunocomplexes of a maximal size of 300kDa, whereas complete anti-TNF-α mAbs form larger complexes of 4000kDa for adalimumab and 14.000kDa for infliximab. The large complexes enhance uptake of the drug to antigen presenting cells (APC), thus increasing immunogenicity, or can directly stimulate B cells in a T-independent way [39].

The entire mAbs may contain multivalent antigens able to induce direct (T-independent) B cell activation through the cross linking of B cell receptors. The mapping of the binding sites of ADA could allow to identify B cell epitopes even though the studies on this issue usually employ linear peptides and not correctly folded mAbs which display the most relevant B cell epitopes [40]. Reported data indicate that the immunogenic region of infliximab lies within the F(ab)2 region, while the humoral immune response to adalimumab is highly restricted to the TNF-α-binding
region [41,42]. Moreover, ADA to adalimumab has been shown to be always neutralizing, whereas different percentages of neutralizing ADA have been reported for other TNF-α blockers; notably the ADA to etanercept are never neutralizing [42–44]. Other biologicals induce ADA with restricted specificity as adalimumab: the anti-OKT3 Abs has been described to elicit only anti-idiotypic and isotopic ADA. By contrast, no anti-allotypic ADA have been described in patients treated with infliximab or adalimumab. On the whole these data indicate the extreme variability of immunogenicity of biologicals to elicit humoral response even if they display specificity for the same target.

After processing ADA/drug immunocomplexes, drug-derived peptides are presented by APC in MHC-restricted manner, thus favoring the recognition and amplification of drug-specific T cell clones. This process is strictly dependent on the numbers and type of immunodominant T cell epitopes available on the drug and on the HLA haplotype of the patient. The study of T cell epitopes includes proliferation assays and cytokine production by freshly isolated mononuclear cells or T cell lines expanded in vitro upon the drug stimulation. The in silico methods is another approach to identify T cell epitopes by predicting the binding affinity of peptides of the entire sequence of biologicals to HLA class I or II [45]. The third approach is the genetic linkage study in which certain HLA haplotypes are linked to ADA outgrowth; a large bulk of in vitro data are now available on the immunodominant epitopes of FVIII which led to the production of a recombinant FVIII molecule (lacking the immunogenic peptides) with reduced immunogenicity in animals. The detection of the immunodominant T cell repertoire of the currently used drugs will help to reduce immunogenicity during the future development of new BA and biosimilars.

**Patient- and treatment-related factors influencing Immunogenicity**

Some patient/disease-related factors contribute to an improved ability to develop the immune response to BA. It is currently known that patients who show immunogenicity to the first TNF-α blocker display an increased risk to produce ADA against also a second anti-TNF-α mAb. In addition, people with high immunoreactivity are more prone to develop ADA [8, 46]. In fact, it has been shown that high expression of costimulatory molecules on dendritic cells in patients with immuno-mediated diseases, may accelerate the development of ADA [8]. Some genetic factors may contribute to immunogenicity, but such a study has low feasibility due to the high number of patients needed. The association between an IL-10 gene polymorphism and ADA development in RA patients treated with adalimumab [47], while others described an association between polymorphisms of IL-10 and TNF-α genes and anti-FVIII ADA detection in hemophilic patients [48]. By a general point of view, the expression of specific HLA haplotypes contributes more easily to ADA development in subjects who do not endogenously exhibit the wild type protein, but display detectable alterations of FVIII-encoding gene.

Also some treatment-related factors may contribute to immunogenicity and ADA production. It is generally accepted that high doses of drug are able to upgrowth tolerance mechanisms and to reduce immunogenicity. A valid drug tolerance is certainly achieved by a long-term regimen and an intravenous-rather than intramuscular- or subcutaneous administration, even though some recent reports do not confirm this view with abatacept or TNF-α blockers [49, 50]. The immunogenicity to biological has been described to be impaired when they are administered in combination with immunosuppressors as mercaptopurine, methotrexate, azathioprine, etc [16, 51-53]. However, we urgently need several controlled studies detecting the prevalence of ADA formation (including defined drug dosages, routes of administration etc) during a combination therapy as well as their increasing risk of diseases related to immunosuppressive therapy. An inverse relationship between the drug dose and its immunogenicity has been reported in patients suffering from RA and Crohn’s disease, indicating that high doses of infliximab are associated with lower incidence of ADA development [23, 52]. However, other reports did not find any significant correlation between infliximab dose and frequency of infusion reactions [22, 23].

**Mechanism of ADA formation**

It is generally accepted that humoral response to therapeutical BA is prevalently due to the activation of an adaptive response to foreign antigens, similar to those against pathogens or vaccines, leading to the expansion of memory T effector and adaptive Treg cells as well as B cells specific for drug dominant epitopes. However, the direct activation of B cells by the drug in a T-independent way or the breakthrough of B and T cell tolerance to autologous proteins present in the majority of treated patients, can contribute in different ways to improve immunogenicity. Indeed, the sequence of events leading to B cell activation and ADA production can follow a T-independent and a T-dependent mechanism. The former occurs when some structural sequence of the drug (namely polymeric repeats or protein aggregates) induces the signals required to directly stimulate B cell subsets. This mechanism is often cited as a source of ADA and usually does not lead to affinity maturation or generation of memory B cells. By contrast, T-dependent B cell activation results in a more robust ADA response, isotype switching and induction of memory B cells. The prevalent induction of IgG class ADA generally implies that biologicals act as T-dependent antigens leading to isotype switch. Of course it also require T cell recognition of immunodominant epitopes in the context of HLA Class I/II molecules of APC and the amplification of T
central and effector memory cells as well as adaptive Treg cells. In the absence of T cell help (CD40L or cytokines) naïve B cells do not mature and antigen-specific B cells become anergic or undergo apoptosis [54]. Therefore the T cell recognition of drug peptides is the prerequisite to generate memory B cells and ADA formation of different isotypes.

The pathogenic mechanism of mAbs-related infusion reaction has not been yet defined, although several mechanisms have been suggested. Pichler proposed that acute adverse side-effects to BA are due to different mechanisms, including hypersensitivity IgE- and non IgE-mediated reactions (type β) and cytokine release syndrome (CRS, type α) [55]. Although IgE-mediated events have a particularly quick and severe onset, usually we are not able to clinically distinguish between IgE- and non-IgE-mediated reactions.

Hypersensitivity reactions have been categorized as type I when mediated by IgE Abs. During the sensitization phase, the first exposure to allergens usually induces Th2 response with a mild production of IgE that bind to specific high affinity Fcε Receptor (FcεRI) on tissue mast cells and circulating basophils [56, 57]. The first exposure to allergen never triggers symptoms, whereas every subsequent exposures do it, as the antigen is recognized by the cell-bound IgE, which stimulates release of histamine and other preformed or neo-formed mediators.

Since the first exposure to antigen is required to induce sensitization, type I hypersensitivity reaction does not occur during the first infusion. However, pre-existing IgE cross-reacting with cetuximab have been detected prior to cetuximab infusion. Anti-cetuximab IgE recognize galactose-α1,3-galactose, an olygosaccharide resulting from a post-translational modification on mAb molecule and sharing some mammalian proteins [58]. Among the cetuximab-treated patients, 32.8% displayed infusion reactions and 68% of them showed pre-existing cetuximab specific-IgE antibodies.

A pre-existing sensitization toward xenogenic murine antigens or additives is a further mechanism accounting for some IgE-mediated reactions in biological-exposed patients. This latter event has been described for polysorbate in patients experienced acute HR to omalizumab [59, 60].

The acute infusion reaction to infliximab is usually accompanied by symptoms suggestive of an anaphylactic reaction [61]. ADA are mostly represented by the IgG isotype [62], even though a proportion of infliximab-related reactions (about 27% of reactive patients) are associated with the presence of IgE isotype mainly with specificity for the idiotype sequences and the murine part of the chimeric drug [24]. The IgE positive patients also resulted positive to intradermal skin test, thus confirming the biological activity of IgE ADA [63]. The atopic phenotype did not apparently affect the incidence of infusion reactions to infliximab and the onset of drug-specific IgE response [22, 64]. Different from the results obtained on cetuximab, a pre-existing sensitization can be excluded for Infliximab. A direct correlation between HR and the presence of serum IgE ADAs has been reported for several mAbs, such as muronomab, cetuximab, tocilizumab and natalizumab [65-68]. Also for rituximab and trastuzumab, the positive results of skin testing supported an IgE-mediated mechanism of HR, at least in some patients [69]. We recently showed that rituximab can induce adverse reactions through an IgE-mediated mechanism, thus suggesting that type I hypersensitivity may be an additional mechanism to CRS in the development of rituximab-related reactions. Also in this patient, we showed the positivity of intradermal test with the drug and a rituximab-specific Th2 response in vitro. Notably the onset of rituximab-specific IgE ADA strictly correlated with the development of the adverse event [70].

The laboratory markers to confirm an IgE-mediated anaphylaxis include the measurement of serum tryptase, the classical mediator accounting for mast cells activation. However, in a cohort of 20 infliximab-reactive patients, serum tryptase levels were found in the normal range after the infusion reaction [71]. However, normal tryptase levels do not exclude IgE-mediated anaphylaxis. It has been found that the majority of patients with fatal or near-fatal food-induced anaphylaxis displayed normal tryptase levels [72]. In these cases, the involvement of basophils has been hypothesized since they express the FcεRI and upon stimulation release mediators such as histamine, but not tryptase. Basophils usually circulate in the bloodstream and are easily triggered by intravenously administered mAbs.

Experimental models revealed that anaphylaxis may occur in mice through two independent pathways. The first pathway involves IgE, FcεRI and mast cells and histamine, while the second one IgG, FcγRIII, macrophages and basophils and the Platelet Activating Factor (PAF) as major mediator [73]. In human anaphylaxis, it has been recently found that PAF levels were directly correlated with the severity of reactions, thus confirming the pathogenic role for mast cell-independent mechanisms [74]. IgG-mediated anaphylaxis seems to be more relevant when large amount of antigen are used and high levels of specific IgG elicited. Whether the murine IgG mechanism is operative also in humans remains at present an open question. Human food anaphylaxis usually involves small amount of allergen and low concentration of antibody, thus suggesting a prevalent IgE-mediated mechanism. However, anaphylaxis occurring during the administration of high amount of immunogenic molecules such as BA may be the exception. Furthermore, IgG ADA have been detected in patients who experienced anaphylaxis during infliximab infusion [62]. Our recent findings displaying some ADA-positive but IgE- and IgM-ADA negative patients with severe infusion reactions, strongly suggest a role for anti-drug IgG isotype as the main
mechanism of anaphylaxis [24]. The development of IgG ADA may activate another effector pathway involving complement activation, production of anaphylatoxins and then a direct not IgE mediated mast cell activation. However, a role for complement in these reactions has not been established until now [75].

Finally a proportion of infusion reactions related to BA (as rituximab) is due to massive cytokine release, leading to an adverse event referred to CRS, that may be indistinguishable from type I hypersensitivity [76]. The symptoms are generally mild to moderate and usually occur within the first couple of hours. Likely the fine mechanism of CRS is related to the cross-linking of mAbs bound to target cells, the complement activation, the lysis of cells and, lastly, to their massive cytokine and chemokines release. Chemokines can recruit effector cells as monocytes, macrophages, NK cells, cytotoxic T lymphocytes, amplifying cells destruction and cytokine release. CRS during rituximab infusion has been observed less frequently in patients with autoimmune disorders than in lymphoma patients likely since in the former it does not cause massive cellular lysis [77, 78]. In contrast to type I HR, CRS may be managed by short-term cessation of the infusion, the administration of anti-histamine drugs and re-starting the infusion at a slower rate [79, 80].

Assessment of Immunogenicity

The immunological response to BA is usually shown by the presence of detectable serum ADA. As previously discussed the prevalence of anti-BA antibody formation, varies widely depending on the type of proteins and the type/phase of patients’ disease, but also of the assay used. Different methods have been reported for the assessment of humoral immunogenicity including double-antigen (bridging) ELISA, sandwich ELISA, radio-immunoassay, surface-enhanced laser desorption/ionization mass spectrometry and surface plasmon resonance [81-84]. Comparison of antibody titres obtained with different tests are at present unlikely as reference units and normal cut-off limits of different assays are not standardized and validated. Because of the difficulties in measuring antibodies and comparing results of different studies, a lot of reports on this topic do not help to understand how, when and why some patients develop antibodies to different proteins [85].

Evaluation of infliximab immunogenicity is of particular interest since sensitivity and specificity of the assays may be influenced by factors including sample handling and timing of sample collection. As known high amount of antigen interferes with commonly used assays by competitive inhibition and/or by forming immunocomplexes and thus, it is highly recommended to sample blood quite far from the infusion taking into account the long half-life of the drug [86]. In order to avoid false negative results, it is mandatory to take sera before the initiation of therapy and immediately before each re-infusion. Upon these experimental conditions, we retrospectively found the presence of serum ADA in patients who have experienced acute infliximab reactions [24]. Furthermore, new immunoassay approaches, such as acid dissociation bridging ELISA, may be envisaged in order to increase the sensitivity of ADA detection [87]. In fact, this new approach allows for detection of antibodies even in the presence of high antigen (BA) concentration [88].

Using ImmunoCAP platform, we and others showed the presence of mAbs-specific IgE antibodies in a proportion of patients with mAbs-related anaphylaxis. The identification of mAbs-specific IgE may be difficult because of the small quantities of this isotype in human serum. In fact, IgE antibodies are usually quantitatively lower than all other isotypes, that may influence IgE assays. To exclude the IgG interference in the detection assay of anti-infliximab IgE antibodies, we showed an increase of ADA IgE after the IgG depletion in some patients, thus suggesting that, at least in some sera, IgG interfere with IgE detection, since they likely share the same specificities.

Since a HR is hypothesized for mAbs, skin tests (prick and intradermal test) with biological agents have been proposed. Positive skin testing are suggestive of an IgE-mediated mechanism as demonstrated in mAbs-reactive patients [24, 69, 89]. However, up to now, no guideline has been proposed to check all patients who had been intolerant to mAbs and skin testing are not done as routine screening [63].

In the last years studies have been addressed to detect memory T cells restricted for MHC class II-restricted epitopes present in mAbs sequences and the subsequent development of humoral immune responses against BA. Evidence that T-cell help is a central component of the pathway that results in ADA formation comes from a number of observations including the prevalence of high affinity responses comprising multiple IgG isotypes [47].

As previously stated the study of memory T cell specific for drug immunodominant epitopes includes proliferation assays and cytokine production by freshly isolated mononuclear cells or T cell lines expanded in vitro upon drug stimulation. Another approach to identify T cell epitopes is in silico method allowing to predict the binding affinity of peptides of the entire sequence of biologicals to HLA class I or II [45]. The third approach is the genetic linkage study in which certain HLA haplotypes are linked to ADA outgrowth. The characterization of eluted peptides directly linked to MHC class II of dendritic cells combined with other proliferative assays allows to map exactly T cell epitopes are presented in vivo inducing T cell response in sensitized/treated patients [90].

In our experience Rituximab-stimulated peripheral blood mononuclear cells (PBMCN) from a reactive patient but not from controls, displayed a dose-dependent proliferative response associated with a Th2 cytokine production profile, able to sustain the IgE...
humoral response [70]. Until now the reports on T-cell epitopes involved in immune responses to TNF-α blockers are rare. This is likely due to the difficulty to distinguish the antigenic from the biological activity of these reagents in vitro [39, 91]. Anyway, preliminary data show that infliximab-specific T cells are exclusively detectable in PBMC of ADA+ reactive patients, whereas memory T cells are virtually undetectable in PBMC of ADA-negative patients (Vultaggio A, unpublished). The presence of mechanisms involving peripheral tolerance such as regulatory cytokines and T cells, able to prevent the formation of ADAs, can be envisaged in ADA-negative patients. Indeed the anti-TNF-α mAbs may di per sè induce reverse signaling on mTNF-α+ cells (activated CD4+ T cells, NK cells and monocytes/macrophages) by promoting silencing signals on some genes and amplifying regulatory mechanisms [91, 92]. One recent study analysed the T cell repertoire specific for some therapeutical BA in healthy donors with the aim to contribute to a better understanding and prediction of immunogenicity; however, these data lack to link the presence of drug-specific naïve T cells in healthy people with the T cell immunogenicity in treated patients [93].

The clinical usefulness of such in vitro and in vivo assays in routine practice remains unexplored. However, monitoring ADA of all isotypes during treatment represents an important issue for the therapy with BA, which allows to predict and reduce the proportion of severe acute reactions. Taking into account that the majority of infliximab reactions could be predicted by the onset of serum ADA, a possible algorithm must include the detection of these antibodies, at least at the beginning of the treatment, when the majority of reactions occur, and in case of re-treatment after discontinuation of therapy. This will allow to identify potentially reactive patients and thus to improve the infliximab safety profile [24].

Risk factors for acute hypersensitivity reactions to monoclonal antibodies

As previously described, the immunogenicity of the drug, and the related onset of adverse acute reactions or LOR, can be due to drug-specific (dose, duration, number of exposures, route of administration) and patient-specific (age, gender, atopic phenotype, and concomitant use of immunosuppressive medications) factors.

Re-exposure following a long interval can trigger a secondary immune response and some reports indicate an increased proportion of BA reactions in patients who have discontinued therapy [94]. Adults and children with Crohn’s disease experienced severe systemic reactions when a distant re-infusion interval was attempted [95]. Several studies suggest that patients receiving regularly scheduled infliximab infusions have a decreased likelihood of forming ADA [14, 89, 96-98]. On the contrary, intermittent therapy may favour ADA formation and increase the likelihood of infusion reaction [18]. More than two/third of infliximab-reactive patients displayed the infusion reaction during the second course of therapy after a variable period of discontinuation [22]. Thus, the breakthrough of tolerance to BA after a period of interruption may be hypothesized. It is likely that regular drug infusion may induce a high-dose tolerance through efficient peripheral mechanisms. Under a certain threshold concentration of circulating BA during treatment or even after a therapeutic interruption, patients may be at high risk of immunization. Results obtained with desensitization for therapeutic mAbs (obtained with gradual re-introduction of small doses of drug at fixed time intervals until the delivery of the therapeutic dose) which are dependent on the ongoing antigen exposure, suggest a role of high-dose tolerance mechanism to BA and the clearcut relationship between infusion reactions and therapy interruption [69].

Conclusions

Advances in the knowledge of pathogenic mechanisms related to immunogenicity of biologicals and of its consequences as loss of response and adverse events have been recently provided. The use of new assays to detect ADA and their isotypes by avoiding the interference of the drug as well as memory drug-specific T cells, may represent an useful tool for monitoring immunogenicity and prevent adverse events. Such a strategy allows also to save resources and may give new insights to the regulatory authorities for the approval of new biologicals or biosimilars. Since the increased use of biological therapies for the treatment of immunomediated disorders and cancer, it is highly advisable that these novel procedures enter into the clinical practice.

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