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The Spectrum and Types of Adverse Side Effects to Biological Immune Modulators: A Proposal for New Classification

(anti-inflammatory therapy / adverse side effects / biological immune modulators / fusion proteins / monoclonal antibodies / tumour therapy)

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Abstract. In recent years, a growing number of biological agents such as cytokines, monoclonal antibodies and fusion proteins have become available for the treatment of various autoimmune, neoplastic, cardiovascular, infectious, allergic, and other conditions. Their introduction has resulted in marked clinical improvements for many patients. Nevertheless, a variety of adverse side effects have been observed with these agents. Based on the special features of biological agents a new classification of these side effects of biological agents is proposed – related but clearly distinct from the classification of side effects observed with chemicals and drugs. This classification differentiates five distinct types, namely clinical reactions due to high cytokine levels (type α), hypersensitivity due to an immune reaction against the biological agents (type β), immune or cytokine imbalance syndromes (type γ), symptoms due to cross-reactivity (type δ), and symptoms not directly affecting the immune system (type ε). This classification could help to better deal with the clinical features of these side effects, to identify possible individual and general risk factors and to direct research in this novel area of medicine.

Introduction

During the last decade many new biological immune modulators ("biological agents") entered the market as new therapeutic principles. They comprise proteins such as cytokines, monoclonal antibodies, and fusion proteins (solubilized receptors). Many of these biological agents have proved to be valuable tools in various inflammatory diseases and tumours, and their direct and focused effect make them superior to immunosuppressive or cytotoxic drugs, whose use is often limited by severe generalized and unwanted side effects. The progress in this field was based on a better understanding of the immunological basis of many diseases, the identification of relevant molecules in inflammation as well as on tumour cells, and the application of biotechnological techniques, which allowed producing recombinant proteins such as cytokines as well as humanized antibodies at a large scale (Abbas and Lichtman, 2005).

The wide use of biological agents in modern medicine is a challenge for physicians, as it is an example of how fast new therapeutical principles based on novel knowledge, and modern techniques can enter clinical practice, and that constant learning is required. Their use often requires a special knowledge and familiarity with the disease to be treated. Moreover, not only the function of these compounds has to be understood, but also the underlying immunology – which is often rather complex. Last but not least, these biological agents are

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Abbreviations : EGFR – epithelial growth factor receptor, IFN – interferon, Ig – immunoglobulin, IL – interleukin, TNF – tumour necrosis factor.

Table 1. Biological agents and drugs: important differences related to adverse side effects (adapted from Weber, 2004)

Biological agent	Drug
Stucturally similar to autologous protein	Synthesized chemicals (xenobiotics)
Digested and proceeded, not metabolized	Metabolized, reactive intermediates with potential immunogenicity (haptens)
Parenteral application required	Oral or parenteral
Immune-mediated effects are inherent in their activity; hypersensitivities are rare and mainly due to immunoglobulins (IgE, IgG)	Immune-mediated side effects are unexpected, differ from the normal action of the drug, and are often T cell-mediated
	Drug interactions, organ toxicity

expensive medicines and force the treating doctors to consider economic aspects as well.

In addition, some concern regards the side effects of these biological agents, which are proteins used like drugs (Weber, 2004). Adverse side effects to drugs are clinically very heterogeneous. One approach was to sub-classify them according to their action: so-called type A reactions correspond to the pharmacological activity of the drug, and are thus predictable (Naisbitt et al., 2000). About 16 % of side effects after drug treatment are type B reactions (Hoigne et al., 1993), which are not related to the pharmacological activity of the drug and are non-predictable. The majority of type B reactions are immune-mediated side effects such as hypersensitivity reactions. Clinically, these immune-mediated side effects are very heterogeneous and can be subdivided according to different pathomechanisms (Pichler, 2003; Abbas and Lichtman, 2005). Biological agents differ from most drugs as they are not small chemical compounds (xenobiotics), but are proteins produced in a way to make them as similar to human proteins as possible (Table 1). They are not metabolized like drugs but are processed like other proteins, and therefore need to be applied parenterally, to avoid digestion in the gastrointestinal tract. Quite a few of them are actually naturally occurring proteins (e.g. cytokines) or humanized antibodies able to neutralize natural proteins. Thus, adverse reactions to biological agents might differ from those elicited by drugs.

The enormous opportunity seen in these molecules and their success in many diseases have led to the generation of many dozens of biological agents in the last years and many more will follow. As these are quite heterogeneous molecules directed to many different structures, it is impossible to cover all adverse side effects in detail. The clear difference of xenobiotics and biological agents with regard to mode of action, chemistry, metabolism, and immunogenicity suggests that a somewhat different approach to their side effects is needed (Table 2). Here, some general aspects of these adverse side effects are outlined, and a new classification for adverse effects of biological agents is proposed. This might help to better understand them and thus to better treat the patient who experiences them. Moreover, it might provide some help to avoid them in the future by defining risk factors and give directions to future research in this novel area.

Table 2. Types of biological response modifiers

Cytokines IFN-α, IFN-ß, IL-2, etc.
Antibodies *To soluble proteins such as cytokines:* Anti-TNF-α (infliximab or adalizumab) Anti-IL-2 (daclizumab) *To cell surface molecules:* Anti-CD20 (rituximab) Anti-IL-2 receptor (basiliximab) Anti-LFA-1 (efalizumab) *To IgE* (omalizumab) *To tumour antigens* (e.g. EGRF, cetuximab, anti-HER2, trastuzumab)
Fusion proteins (soluble receptors for cytokines or soluble cellular ligands) TNF-αRII (etanercept), a soluble TNF-α receptor CTLA4-Ig (abatacept) blocking CD28-CD80/CD86 interaction IL-1 receptor antagonist (anakinra)*

Abbreviations
* Not a fusion protein, but acting in a similar way

Biological agents

The biological agents on the market or in clinical trials are mainly tools to affect inflammatory processes and malignancies. They can be subdivided into the following classes (Table 3).

Cytokines

Cytokines such as interferon-alpha (IFN-α), interferon-beta (IFN-β), and interleukin 2 (IL-2), etc., are widely used biological agents. Some of these cytokines have been modified to prolong their *in vivo* half life (containing polyethylene glycol, which reduces degradation, e.g. peg-IFNs). Their amino-acid sequence is identical to human proteins but their glycosylation might differ.

Table 3. Side effects of IFN-α and anti-TNF-α

	IFN-α	Anti-TNF-α (infliximab)
Type α: High dose	Flu-like symptoms Myalgia Arthralgia Fever	-
Type ß: Hypersensitivity	Local and generalized urticaria Local dermatitis	Local and systemic urticaria, erythema, serum sickness Loss of efficiency
Type γ: Immune/cytokine imbalance syndromes		Acute and delayed infusion reactions, local dermatitis
Immunodeficiency	-	Tuberculosis, listeriosis, other granulomatous infectious diseases Demyelinating diseases, pancytopenia, lichenoid skin reaction, psoriasis
Autoimmune/autoinflammatory disorders Thrombocytopenia, haemolytic anaemia	Intesticial pneumopathy, acute fibrosis, systemic sclerosis, SLE, IgA nephropathy, dermatitis herpetiformis, SLE, vasculitis, thyroid disease, pernicious anaemia sarcoidosis, psoriasis, vitiligo	
Atopis-allergic	-	Atopic dermatitis
Type δ: Cross-reactivity	-	?
Type ε: Non-immunological side effects	Neurological symptoms such as Bell's palsy, hearing loss, depression, dystonia, restless legs	Heart insufficiency

Abbreviations
SLE - systemic lupus erythematodes, ? - unknown

Antibodies

Xenogeneic antibodies (e.g. from horse) were already in use at the beginning of the last century and were ominous for causing severe hypersensitivity reactions such as anaphylaxis and serum sickness after repeated injections. The development of monoclonal antibody by *in vitro* technology led to a revolution in this field as it allowed simplified generation of antibodies directed against a specific surface molecule, a soluble molecule, a cytokine, etc. (Abbas and Lichtman, 2005). While the original monoclonal antibodies used for therapeutic purposes were of mouse origin, the progress of molecular biological techniques allowed their modification, and thus the majority of antibodies now in use are chimaeric, humanized, or fully human antibodies. The chimaeric antibodies like "infliximab" are characterized by the postfix "ximab", while humanized antibodies like "daclizumab" or "omalizumab" carry the postfix "zumab", and fully human antibodies like "adalimumab" carry "mumab".

- Anti-cytokine antibodies consist of antibodies directed to cytokines, e.g. anti-IL-5 or anti-TNF-α antibodies.
- Antibodies blocking cell-bound molecules such as adhesion molecules, e.g. efalizumab, an anti-LFA-1 antibody, or an anti-IL-2 receptor antibody (basiliximab or daclizumab).
- Antibodies with the ability to deplete or inactivate certain cells; e.g. anti-CD20 antibodies (rituximab) or some antibodies directed against tumour antigens. Some activity might be due to down-regulating the target structure on the cell, thus inactivating it (Walker et al., 1989). Others might even transiently activate the target cell (anti-CD3 antibodies, muromunab).

Fusion proteins

Natural receptors have often a very high affinity for their ligands and are thus as potent as high-affinity antibodies. To solubilize and increase the half-life of these normally cell-bound molecules, they are fused with the Fc part (CH2, CH3) of human immunoglobulin IgG1. A special case is represented by the naturally occurring soluble IL1 receptor antagonists (anakinra) (Waugh and Perry, 2005).

Soluble cytokine receptors are named using the ending -cept, like in etanercept (the p55, soluble tumour necrosis factor-α receptor II (TNF-α RII)).

Soluble cell ligands interfere with the cell-to-cell communications. To block this interaction, either antibodies to the ligand or a soluble form of the ligand itself can be used to interfere. Thereby co-stimulation of cells or their migration can be blocked (the interaction of CD28 or CTLA4 (on T cells) with CD80/CD86 on antigen-presenting cells (Table 3). This interaction can be blocked by CTLA4-Ig (abatacept), which is a fusion

protein, between CTLA4 (expressed on activated T cells) and IgG1-Fc; CTLA4 has a 10-fold higher affinity for CD80/CD86 than CD28 and is thus able to block the interaction of CD80/CD86 with CD28 on T cells (Abbas and Lichtman, 2005).

General principle of adverse effects of biological agents

In a recent review of adverse reactions of biological agents, Lee and Kavanaugh differentiated between target-related or agent-related adverse side effects (Lee and Kavanaugh, 2005). Indeed, target-related side effects are common with biological agents, as e.g. a biological agent may alter the composition and functional integrity of the normal immune response, and thereby predispose the patient to certain side effects, while the agent itself is rather harmless. Consequently, to understand side effects of biological agents, one has to be aware of the activity of the biological agents.

- Many biological agents have a well-defined range of physiological actions (Abbas et al., 1996), some of which may already explain some adverse side effects. It is expectable that high concentrations of a pro-inflammatory cytokine such as IFN-α can cause symptoms that are also observed during an immune reaction with a high IFN-α level (e.g. flu-like syndrome).
- Biological agents often affect T and B cells or their products, as well as the different effector cells leading to various forms of inflammation. Side effects of biological agents affecting the immune response are to a certain degree predictable and consequently cannot be classified as unpredictable "type B" reactions (Naisbitt et al., 2000). In the same line can a hypersensitivity reaction to an injected protein containing parts of a foreign protein be classified as an unpredictable type B reaction, or being actually a predictable reaction.
- Immunological reactions during therapy with small molecular compounds (drugs) are mainly classified as hypersensitivity reaction (Pichler, 2003). Hypersensitivity reactions are immune responses against the substance applied, which surely does not explain many of the side effects seen with these biological agents.

The many distinct functions of these biological agents make it impossible to sub-classify their adverse side effects based on clinical symptoms. More appropriate is a sub-classification based on the mechanism of action and structure, as illustrated in Fig. 1. To distinguish it from the classification of side effects to chemicals/drugs, the Greek alphabet is used for the five types (type α, β, γ, δ, and ε, Fig 1).

- Type α (high cytokine and cytokine release syndrome): Side effects might be connected to the systematic application of cytokines in relatively high doses or to high concentrations of cytokines released into the circulation (Vasquez et al., 1995).
- Type β (hypersensitivity): The second group of reactions can be termed as "hypersensitivity". Thereby, basically three forms of allergies can be differentiated: IgE-, IgG-, and T cell-mediated reactions.
- Type γ (immune (cytokine) imbalance syndromes): A major group of side effects have immunological features, but cannot be explained by high cytokine levels or typical hypersensitivity reactions. As illustrated in Fig. 1, these reactions can be further sub-divided into *impaired function*, and *unmasking* or *causing* an *immune imbalance* leading to *autoimmune*, *auto-inflammatory* or *allergic reactions.*
- Type δ (cross-reactivity): Another cause for side effects might be that antibodies generated to an antigen expressed on tumour cells might also cross-react with normal cells, which also express this structure, albeit to a lower degree (Peréz-Soler and Saltz, 2005).

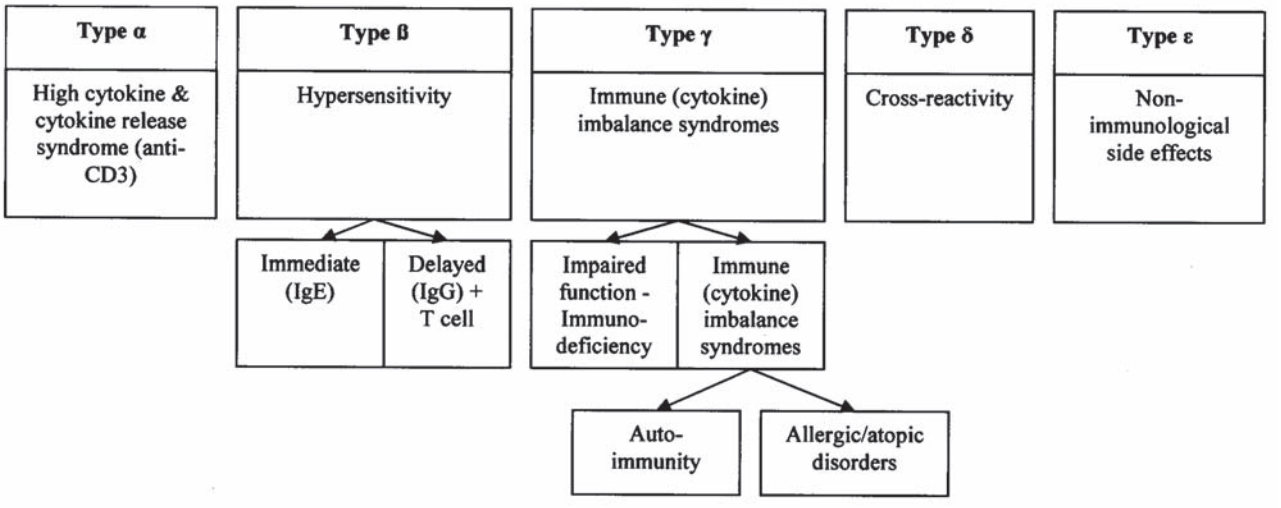

Fig. 1. Type of adverse side effects to biological agents.

- Type ε (non-immunological side effects): Quite a few of the biological agents may elicit symptoms not directly related to the immune system, sometimes revealing unknown functions of the biological agents given or targeted.

This classification considers the well-accepted classification of side effects to drugs, as the first two types are similar (type A/α are both dose-dependent and related to the function of the drug or biological agent; type B/β comprises hypersensitivity). One has, however, to emphasize that it is not a clinical classification based on similarity of symptoms, but an attempt to classify the side effects according to mechanism.

Type α - high cytokine and cytokine release syndrome

Most cytokines (as well as chemokines) are produced locally and have predominant local activity: their action is directed to the neighbouring cell (paracrine) or has even an autocrine function (Abbas and Lichtman, 2005). Some cytokines, e.g. TNF-α or IL-5, also have a systemic activity, which comes into play if the immune reaction is strong and a systemic reaction of the immune system required (Abbas and Lichtman, 2005). Thus, for most cytokines only the local concentration is relatively high, while the systemic concentrations are rather low and often affect bone marrow-derived progenitor cells. If the cytokine is applied therapeutically, the situation is inverse; comparatively high systemic concentrations are applied to achieve a sufficiently high concentration locally. Such high systemic concentrations can sometimes cause severe, not tolerable side effects, limiting the use of cytokines (fever, myalgia, headache, etc.). Alternatively, one of the first monoclonal antibodies on the market was directed against CD3 (muromunab), which is the signal transmitting complex associated with the specific T-cell receptor for antigen. Cross-linking these T-cell receptor-associated molecules leads to activation of T cells and release of different cytokines into the circulation with generalized symptoms such as flash, arthralgia, capillary leak syndrome with pulmonary oedema, encephalopathy, aseptic meningitis, pyrexia, gastrointestinal symptoms such as severe vomiting or diarrhoea, called cytokine released syndrome (Vasquez et al., 1995).

Type β - hypersensitivity reaction to biological agents

Different factors determine the immunogenicity of the biological agents, and the type of clinical symptoms because of real hypersensitivity.

Degree of humanization: Allergic reactions to biological agents are directed against the protein itself. The frequency of such reactions depends on the degree of humanization of the applied protein, which is often an antibody. The allergic immune response can be directed to the constant or variable part. While e.g. mouse antibodies (almost not used any more) and chimaeric antibodies have at least some xenogenic determinants on their constant part, which can elicit an immune response quite rapidly, humanized or fully human antibodies have a low immunogenicity as immunological tolerance exists to the constant part of the immunoglobulin. Nevertheless, the antigen-binding site of the monoclonal antibody can still elicit an immune response (anti-idiotypic) (Chatenoud, 1993).

Co-factors: Another important aspect for the immunogenicity of a biological agent is its content of adjuvant. For the cases of pure red cell aplasia observed in 2000 and 2001 outside the USA and related to erythropoietin injections, differences in rubber stoppers used for vials containing the erythropoietin is thought to have contributed to the immunogenicity, as certain stoppers allowed the diffusion of some organic compounds with adjuvant activity inside the vial, which was enough to cause immunogenicity of the erythropoietin (Boven et al., 2005). The way of application (s.c. vs i.v.), the IgG isotype of the biological agent, and in particular the amount of immunosuppressive co-treatment, may also have an influence. For example, the sensitization and antibody formation to infliximab, a chimaeric anti-TNF-α antibody, can be reduced by co-medication with methotrexate (Lipsky et al., 2000; Baert et al., 2003).

Type of allergic reaction: The IgE-mediated reactions can cause a local wheal and flare reaction at the injection side when applied s.c., but may also cause urticaria anaphylaxis. Such a reaction appears rather rapidly, that means within 20 minutes after the injections. One has to differentiate it from an unspecific irritation induced by the solvent, which may also lead to local redness and a typical wheal. Irritative responses are often diminished at subsequent applications – but this is not a strict criterion to differentiate it from real allergy, as tolerance might develop in IgE-mediated reactions. The majority of these allergic reactions are mild, but severe IgE-mediated anaphylaxis has also been described (Abramowicz et al., 1992). Acute infusion reactions are mostly not IgE-mediated. They occur in 3–5% of patients treated with chimaeric antibodies, often already during the infusion, and can be reduced by slowing the infusion rate (Han and Cohen, 2004). Their pathomechanism is unclear, but may be related to activation of cells (by Fc-IgG receptors) or of the complement system via immune complexes, as they appear more frequently when antibodies are detectable (Baert et al., 2003; Arimura et al., 2004; Han and Cohen, 2004). Symptoms are chills, nausea, dyspnoea, headache, and fever (Han and Cohen, 2004).

Delayed reactions appear > 6 h after the application. They can be subdivided into immunoglobulin- and T cell-mediated reactions. The normal physiological im-

mune response to a foreign, soluble protein is immunoglobulin-mediated. Thus, the development of IgG antibodies directed to the biological agent is by far the most frequent reaction. Formation of IgG antibodies against the biological agent may occur rather frequently, if the biological agent is immunogenic and if no immunosuppression, e.g. methotrexate, accompanies the treatment (Baert et al., 2003). In the study with infliximab, up to 68 % of the treated patients developed antibodies to this chimaeric antibody (Lipsky et al., 2000; Baert et al., 2003). These antibodies are not necessarily associated with symptoms. The most frequent effect is inactivation of the biological agent. The half-time of an injected cytokine or antibody is reduced and the patient needs more of the biological agent or an alternative to achieve the same effect. However, the substance injected is unique for a certain function; the inactivation may have severe consequences. This has been shown for anti-erythropoetin antibodies, which led to pure red cell aplasia (Boven et al., 2005). Formation of antibodies to the biological agent may also result in activation of the complement cascade via immune complex formation as well as by FC-IgG receptor-mediated activation of the neutrophils and may thus cause immune complex diseases such as serum sickness, vasculitis and nephritis. Some symptoms appear after 3 to 12 days, and are classified as delayed infusion reactions, characterized by myalgia, arthralgia, fever, "rash", pruritis, facial and lip oedema, dysphagia and urticaria (Han and Cohen, 2004). Another immunoglobulin-associated side effect may be thrombocytopoenia, if immune complexes are formed that bind to Fc-IgG receptors on thrombocytes, which are then removed from the circulation by the phagocyte system in liver and spleen (Arimura et al., 2004). In these immunoglobulin-dependent reactions T cells are probably also involved, but mainly as regulators of the humoral immune response. In contrast to hypersensitivity reactions to small-molecular-weight compounds (chemicals/drugs), where T cell-mediated reactions cause different forms of exanthemas or hepatitis, etc. (Pichler, 2003), biological agents seem to elicit such reactions quite rarely. However, immunohistological examinations of delayed appearing and persisting injection site reactions to etanercept (soluble TNF-α R) revealed infiltration of T cells (Werth and Levinson, 2001), suggesting that T-cell reactions themselves may cause clinical symptoms. If a hypersensitivity reaction is suspected, one can confirm it by skin tests with the biological agents; if specific IgE to the biological agent is present, a local wheal and flare reaction might appear; if T cells are involved, indurations and vesicle formation can be seen after 24–72 h. Alternatively, enzyme-linked immunosorbent assays (ELISA) detecting newly formed antibodies to the biological agent (e.g. human anti-chimaeric or anti-mouse-immunoglobulin antibodies.) can confirm the presence of antibodies.

Type γ - immune/cytokine imbalance syndromes

Quite a few side effects to biological agents cannot be explained by high concentrations or by an immune response directed to the biological agent and thus are not hypersensitivities. Tests to detect hypersensitivities, e.g. skin tests with the biological agent, as well as *in vitro* determination of antibodies to the substrate are negative. Some side effects might be explained by the potent and unique activity of the biological agent in certain types of the normal immune response or by the elimination of certain cytokine activity by an injected antibody. Other effects are often not explainable as easily. They may reveal a new or neglected activity of the biological agent given or eliminated. Thereby, the broader the physiological role of the biological agent, the more heterogeneous effects can be seen. For example, recombinant erythropoietin or omalizumab, an antibody directed against IgE, have a limited pattern of adverse side effects, as they replace or reduce a certain effector molecule with a limited function in the immune system. In contrast, essential, broadly active cytokines such as IFN-α or TNF-α are associated with a wide variety of quite different side effects, due to the very broad activity of these cytokines (Kassiotis and Kollias, 2001; Vermeire et al., 2003; Banchereau et al., 2004; Weber, 2004).

Impaired function (immunodeficiency)

Quite a few of the biological agents are actually used in inflammatory disorders or transplantation, and one aim of the treatment is to dim the inflammation or immune response to the transplanted organ (Chatenoud, 1993; Herschbergerger et al., 2005). The best understood and to a certain extent expected adverse side effect of certain biological agents is impaired function of the immune system resulting in a certain immunodeficiency. Actually, one could also classify impaired function as predictable, type α reaction. However, type A/α reaction is mainly due to a high dose, which may or may not lead to immunodeficiency, and therefore impaired function is classified within imbalance syndromes. Typical examples would be efalizumab, an antibody to LFA1 (CD11a), the ligand for CD18 on neutrophils and T cells. It inhibits the migration of these cells into the affected tissue (Herschbergerger et al., 2005). While this may be beneficial for example in psoriasis, it may be contra-productive for the optimal and rapid control of infections. TNF-α is another example; one main obstacle in the use of anti-TNF-α therapy is the danger that an underlying disease such as tuberculosis or listeriosis escape the control of the immune response and disseminate, as TNF-α is essential for the control of these intracellular infections by stimulating macrophage function (Weber, 2004; Wellington and Perry, 2005).

Unmasking a pre-existing imbalance or causing an imbalance

The immune system is well balanced, and the central and peripheral tolerance mechanism, regulatory T cells, certain cytokines, e.g. transforming growth factor (TGF) β and IL 10, as well as the Th1/Th2 balance are involved (Abbas et al., 1993; Kassiotis and Kollias 2001; Banchereau et al., 2004). A disturbance of this balance can occur by eliminating or injecting certain cytokines that have an immunoregulatory function. It can result in autoimmunity (e.g. systemic lupus erythematodes) and auto-inflammatory responses (e.g. eosinophilic or neutrophilic inflammations without auto-antibodies, e.g. psoriasis), if the immunological tolerance to auto-antigens is altered. It might also lead to the appearance of other immunological reactions, which are normally suppressed, e.g. an immune response to a harmless exogenous antigen (allergic and atopic disorders). All three patterns have been described for anti-TNF-α, IFN-α, anti-CTLA4-antibodies and others (Kassiotis and Kollias, 2001; Debandt et al., 2003; Gomez-Reino et al., 2003; Phan et al., 2003; Vermeire et al., 2003; Seckin et al., 2004).

Autoimmunity and auto-inflammatory disorders

Tumour necrosis factor-α neutralization leads rather frequently to auto-immune phenomena and rarely even to auto-immune diseases. Anti-nuclear antibodies can be found in up to 11% of patients treated with etanercept, a soluble TNF-α receptor (Day, 2002), and in up to 68% in patients treated with infliximab (Lipsky et al., 2000; Baert et al., 2003). However, the development of clinical lupus is a rather rare event (approximately 0.5% of cases) (Debandt et al., 2003; Weber, 2004). Also the development of demyelization diseases have been observed under anti-TNF-α treatment (Day 2002), and treatment of patients with multiple sclerosis with lenercept (the soluble, p75 form of the TNF-α RI) had to be stopped, as the disease became more severe (Weber, 2004). The reason for this stimulation of autoimmune reactions by this presumably immunosuppressive treatment is unclear, but deregulated TNF-α has been associated with autoimmunity (Kassiotis and Kollias, 2001; Banchereau et al., 2004). Interestingly, the use of an immunostimulatory cytokine like IFN-α treatment may also induce autoimmune and auto-inflammatory diseases, as lupus-like syndrome, systemic sclerosis, Guillain-Barré syndrome, autoimmune thyroid disease, idiopathic thrombocytopoenic purpura, vitiligo, and psoriasis have been described (Arimura et al., 2004; Boz et al., 2004; Seckin et al., 2004; Solans et al., 2004; Doi et al., 2005; Niewold and Swedler, 2005). The underlying pathomechanism is not yet understood; it could be due to an immunostimulatory effect of IFN-α leading to the appearance of hidden antigens, the enhanced expression of co-stimulatory molecules, or to enhanced signalling in activated B cells secreting auto-antibodies (Rifkin et al., 2005). Auto-inflammatory of allergic diseases may also arise if a shift of the Th1-Th2 balance, which regulates the type of immune response, occurs. Th1 cells booster macrophage function, production of complement-fixing antibodies and cellular immune responses, while Th2 cells enhance production of IgE/IgG4 and eosinophilic inflammations. Both T-cell subsets also control each other, as e.g. the Th2 cytokine Il-4 downregulates Th1-driven macrophage functions, but boosters IgE responses, while the Th1 cytokine IFN-γ stimulates macrophages but can suppress IgE (Abbas and Lichtman, 2005). Biological agents can interfere with this balance, e.g. a Th1-driven auto-inflammatory process might be enhanced by IFN-α or be dimmed by reducing the high activity of e.g. TNF-α. While the suppression of TNF-α may give a rather good result in the control of an auto-inflammatory process such as rheumatoid arthritis or Crohn's disease, it may uncover a controlled readiness to generate a Th2 response (Devos et al., 2003; Phan et al., 2003; Chan et al., 2004; Menon et al., 2004).

Atopic/allergic disorders

The immune response to harmless exogenous antigens is normally suppressed, but under certain circumstances these tolerance mechanisms fail and atopic/allergic diseases may develop (Taylor et al., 2004). With anti-TNF-α treatments, various skin diseases appeared, and some had the clinical features of atopic dermatitis. This could reflect development of a Th2-biased disease due to suppression of TNF-α, whereby it is unknown whether exogenous or auto-antigens drive this reaction (Devos et al. 2003; Chan et al., 2004). Abrogating the suppressive function of activated CTLA4+ T cells, which have immunoregulatory properties, by anti-CTLA4 antibodies (MDX-010) may also lead to skin symptoms, e.g. eosinophilic dermatitis similar to drug hypersensitivity (Phan et al., 2003). The immune imbalance syndromes are clinically very heterogeneous, dependent on the effect of eliminating or bolstering a crucial cytokine or function/expansion of a cell. They occur only in a minority, suggesting that either the individual predisposition or an individual co-morbidity may be important for the treatment to result in a clinical symptom. These immune or cytokine imbalance syndromes are complex diseases and surely need to be better defined for each biological agent, which may reveal interesting, neglected aspects of the target molecule and pave the way to identify individuals at risk.

Type δ - cross-reactivity

Cross-reactivity can be due to expression of the same antigen on different tissue cells or reaction of the antibody with a similar structure. Tumour antigens are often "normal" proteins, which are over-expressed on tumour

cells. Antibodies to these antigens may also react with these structures on normal cells; e.g. epidermal growth factor receptor (EGFR) is strongly expressed on a variety of carcinomas of different origin and is thought to be partly associated with tumour progression (Peréz-Soler and Saltz, 2005). In addition, EGFR plays a major role in the homeostasis of the epidermis and epidermal appendages. Antibodies to these EGFR (e.g. cetuximab) are used in the treatment of various tumours. Interestingly, acneiforme eruptions appear very frequently in the frame of these anti-EGFR treatments; possibly due to cross-reactivity with EGFR on skin cells (Peréz-Soler and Saltz, 2005). Similarly, it cannot be ruled out that some of the antibodies used also react with structurally similar proteins, and thus cause unexpected side effects.

Type ε - non-immunological side effects

Many molecules, originally detected in the immune system and inflammatory response may also be involved in other physiological functions. Actually, the *in vivo* use of a biological agent in humans may reveal these "new" functions; e.g. blocking CD40-CD40-ligand interactions (important for immunoglobulin class switch in B cells), where both soluble CD40-Ig or anti CD40L antibodies precipitated the appearance of thrombosis and subsequently the detection of the CD40 and CD40L on thrombocytes (Danese and Fiocchi, 2005); or the role of TNF-α in heart failure, where high TNF-α levels were detected, but neutralization of TNF-α led to aggravation of the disease (Kwon et al., 2003). Also the rather frequent neuropsychiatry adverse effects of IFN-α (acute confusional state, depression), as well as various retinopathies observed during IFN-α treatments may represent such type ε reactions (Kwon et al., 2003; Kasahara et al., 2004). Manifestations of such non-immunological side effects might actually be quite frequent. Some of these type ε reactions may be due to cross-reactivity (type δ reactions), if antibodies are involved. On the other hand, such unexpected side effects of biological agents provide a chance to detect new functions of molecules which were originally detected in the immune response, but play a role outside it as well.

A further aspect to be considered in the evaluation of side effects in the combined use of biological agents and drugs, e.g. treatment of hepatitis C infection, where IFN-α is often provided in combination with ribavirine; if anaemia develops, it might be related to ribavirine, while the development of autoimmunity is likely due to IFN-α itself (Bagheri et al., 2004; Chamberlain and Poon, 2004). And in oncology, where many biological agents are in use, attempts are made to increase the efficacy of the treatment by coupling cytotoxic or radioactive compounds to biological agents, which of course can be responsible for adverse side effects (Panwar et al., 2005).

Conclusion

Biological agents are used like drugs, but they have many features which distinguish them from drugs, and this has important consequences for understanding and classifying adverse side effects. As biological agents will be used far more in the future, it is essential that the knowledge about these adverse side effects is improved. An analysis of the adverse side effects of different biological agents reveals that many are related to their biological activity and are not due to an immune response against them, as it occurs in hypersensitivity. Based on these observations a new classification of these adverse side effects of biological agents is proposed – related but still distinct from the classification of side effects observed with chemicals used as drugs. It is clear that such classification based on the mechanism of the biological agent needs to be evaluated in the daily care of patients treated with the biological agent and thereby prove its practicability. This will reveal whether it is too complex or fulfils all or at least some of its scope, namely to help better understand the clinical features, to direct research in this area, and possibly identify individual and general risk factors, which would reduce the incidence of adverse side effects.

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