Biologics

11

Abstract

Currently, ~28–30 mAbs are approved or under consideration for approval as specific therapies in the USA or European Union, although about 350 new mAbs for therapeutic application in humans are in the commercial pipeline. So far, the number of target antigens for the mAbs is surprisingly small with more than one of the approved antibodies specific for TNF, HER2, CD20, EGFR, or VEGF. Other specificities include EpCAM, glycoprotein IIb/IIIa, CD30, CD52, C5, α-4 integrin, IgE, IL-6R, BLys, IL-1β, and RANK-L. Initial infusion reactions to some mAbs may provoke tumor lysis syndrome, cytokine release syndrome, and systemic inflammatory response syndrome. Systemic and cutaneous reactions also occur to mAbs. Rituximab, for example, may cause serum sickness, vasculitis, cutaneous reactions, interstitial pneumonitis, and ARDS as well as post-infusion reactions. Some patients receiving cetuximab experienced severe immediate hypersensitivity reactions. The antibodies involved are IgE specific for α-D-galactose-(1–3)-β-D-galactose and reactive with this disaccharide present on the Fab portion of the chimeric antibody. The nature of, and main adverse reactions to, etanercept, the synthetic IFNs pegylated IFNα-2a and pegylated IFNα-2b, IL-2, denileukin diftitox, anakinra, aflibercept, anti-thymocyte globulin, epoetins, and recombinant human insulin are discussed.

Until the late 1990s, the backbone of treatments for malignancies was the administration of cytotoxic chemotherapeutic agents. However, from about 1998 and with ever expanding usage over the last 14 years, passive immunotherapy with targeted agents, generally monoclonal antibodies, is being used to manage various human cancers, some autoimmune diseases such as rheumatoid arthritis and Crohn's disease, some cardiovascular diseases, systemic lupus erythematosus, asthma, and for the prevention of organ transplant rejection. This new class of therapeutic drug, often referred to simply as "biologics," encompasses an expanding diversity of agents, many of which are genetically engineered copies or modifications of natural products of the body's immune system. Biologics may be composed of proteins, nucleic acids, sugars, or combinations of these; they may

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be of human, animal, or microbial origin; and, in the vast majority of cases, they are created by biological processes, or biotechnology, rather than chemical synthesis. Besides recombinant therapeutic proteins such as some antibodies, cytokines, and receptors, included in any list of biologics would be gene therapies, somatic cells, adult and embryonic stem cells, vaccines, tissues, and blood products and components.

11.1 Monoclonal Antibodies for Therapy

Monoclonal antibodies (mAbs) for the treatment of diseases belong to a new class of therapeutic agents called biologics. From their announcement in the scientific literature in 1975 and for many years thereafter until more recent times, these antibodies have been produced using mouse hybridoma cells prepared by fusing spleen cells from an immunized mouse with mouse myeloma cells. The hybridoma cells so produced retain the capacity to make specific antibody while the myeloma cells impart the capacity of the cells to grow indefinitely in culture, continuously secreting antibody. For therapeutic use in humans, the mouse antigens rapidly induce an immune response which not only inhibits the mouse antibody's action but can also provoke allergic reactions including anaphylaxis. This has led increasingly to the development of methods to humanize mAbs. One approach involves the production of chimeric antibodies by splicing the variable regions encoding the antigen-recognition determinants from a mouse antibody into the constant and Fc regions of human IgG. While this is one step forward in attempts to eliminate or attenuate an immune reaction, mouse antigens in the variable regions can sometimes still stimulate an immune response. Another approach involves the creation of a phage display library by the fusion of gene segments encoding human antigen-binding variable regions to genes encoding bacteriophage protein coat. Newer methods for the production of human mAbs utilize transformed human B cells, plasmablasts secreting antibody, or by generating human B cell hybridomas.

11.1.1 Nomenclature

The nomenclature of monoclonal antibodies is the one used by both the U.S. Adopted Names (USAN) and World Health Organization's International Proprietary Names (INN) for pharmaceuticals. All of the monoclonal antibody names end with the stem *-mab* and use preceding substems depending on the structure and function of the antibodies. Different modified suffixes or stems are added to distinguish the origins of mabs. Those of murine origin are designated by the stem *-omab*; chimeric antibodies in which the variable region is spliced into a human constant region are given the *-ximab* stem; humanized antibodies with the murine hypervariable regions spliced into a human antibody have the *-zumab* stem and antibodies with a complete human sequence are given the *-mumab* or *-umab* suffix.

11.1.2 Monoclonal Antibodies Approved for Therapy

Only 11 years after the publication of Köhler and Milstein's paper on the development of hybridoma technology, the first therapeutic monoclonal antibody OKT3 (muromonab, Orthoclone) received regulatory approval. Over the last 15 years, specially designed and produced mAbs have become one of the most important and successful therapies for patients with hematological malignancies and solid tumors. These targeted agents are also finding increasing application for the treatment of other diseases such as chronic asthma, psoriasis, systemic lupus erythematosus, and macular degeneration; the prevention of the rejection of transplanted organs and graft-versus-host reactions; inhibition of platelet aggregation in some cardiovascular diseases; treatment of autoimmune diseases such as Crohn's disease and rheumatoid arthritis; for paroxymal nocturnal hemoglobinuria and cryopin-associated periodic syndrome; and for inhibiting respiratory syncytial virus. Most are produced in Chinese hamster ovary cells, Sp2/0 cells, or NSO cells. The subclass of immunoglobulin used in therapeutic mAbs is an important consideration, especially in

treating tumors. A glance at a list of mAbs shows that IgG1 is frequently selected, IgG2 and IgG4 are occasionally employed, and IgG3 is rarely if ever used. This relates to the different biological properties of the antibody subclasses—IgG1 is the subclass of choice for antibody-dependent cell-mediated cytotoxicity which makes it eminently suitable for treating cancer cells while IgG4, which does not aid cytotoxicity, is the choice when cell killing is not wanted. IgG3 is seldom used since it has a significantly decreased half-life.

At the time of writing, the ~28–30 mAbs approved or under consideration for approval as specific therapies in the USA or European Union are listed in Table 11.1. The great majority of these mAbs are for cancer immunotherapy. Humanized and human monoclonal antibodies each comprise about one-third of the approved total. Two of the antibodies are administered with radiolabels, ibrutumomab tiuxetan with yttrium-90 or indium-111 and tositumomab with ^{131}I; catamaxomab has dual specificity, for EpCAM (epithelial cell adhesion molecule) and CD3; and only one, brentuximab vedotin, is an antibody–drug conjugate. However, it is true to say that the number of target antigens is surprisingly small with more than one of the approved antibodies specific for TNF, HER2, CD20, EGFR, or VEGF. It is said that about 350 new mAbs for therapeutic application in humans are in the commercial pipeline and it is likely that many of these will be antibody–drug conjugates, bispecific, and/or specifically engineered fragments or domains. There may be at least some truth in those who predict that the future of therapy belongs to the emerging biologics.

11.1.3 Immune Reactions to Monoclonal Antibodies

Reactions, both immune and innate and to human as well as foreign proteins, may occur to mAbs. Acute reactions caused by a number of different mechanisms have been reported. These reactions include true, type I anaphylaxis, delayed reactions, anaphylactoid responses, serum-sickness-like reactions, cytokine release syndrome, and tumor lysis syndrome (refer Sect. 13.5). The range of clinical manifestations include those seen in local skin reactions at the injection site through to cutaneous and systemic hypersensitivities and sometimes pyrexia, an influenza-like syndrome, and the potentially fatal systemic inflammatory response syndrome. Acute anaphylaxis and anaphylactoid reactions are well known to occur with mAbs. Anaphylaxis to some mAbs such as cetuximab and omalizumab has been frequently described and is of special interest (see below), but there are reports of reactions to others including muromonab, basiliximab and OKT3. Incidences of immediate hypersensitivity have been reported for infliximab (2–3 %), rituximab (5–10 %), trastuzumab (0.6–5 %), omalizumab (0.1–0.2 %), and from <1 to 3 % for cetuximab with much higher rates reported in some regions of the USA (see Sect. 11.1.3.2). Serum sickness has been described for a number of mAbs including the chimeric antibodies infliximab and rituximab and the humanized mAbs alemtuzumab and natalizumab. For both the initial reactions and overall, cutaneous reactions are the most frequently seen adverse responses to mAbs.

Reactions following initial infusions of antibody are common, but these can usually be handled by a cautious rate of infusion, appropriate hydration and diuresis, and, if necessary, premedication. Twenty six percent of initial reactions are reported to be mild, 48 % moderate, and 26 % severe. The initial infusion reaction to some mAbs, for example, rituximab (see below), may provoke tumor lysis syndrome, cytokine release syndrome, and systemic inflammatory response syndrome. Tumor lysis syndrome, noted particularly with rituximab, can occur following cancer treatment and sometimes without treatment. It is believed to be the result of breakdown products of cancer cells leading to increased levels of some metabolites and reflected in conditions such as hypercalcemia, hyperkalemia, hyperphosphatemia, acute uric acid nephropathy, and acute renal failure. The syndrome can occur in the early stages of mAb therapy and is potentially life-threatening. Cytokine release syndrome, also called cytokine storm, is commonly seen after

Table 11.1 Therapeutic monoclonal antibodies (mAbs) marketed or under review in the USA or European Union (As at June 2012)

Generic name	Type of mAb	Cell line	Target[a]	Mechanism of action	Approved indication	Trade name
-omabs						
Catumaxomab	Rat IgG2b/Mouse IgG2a bispecfic	Hybrid hybridoma	EpCAM/CD3	Binds both EpCAM and CD3	Malignant ascites	Removab®
Ibritumomab tiuxetan	Murine IgG1κ	CHO	CD20	Binds malignant B cells[b]/ADCC[c] and CDC[c]	Non-Hodgkin lymphoma	Zevalin®
Tositumomab-^{131}I	Murine IgG2aλ	Hybridoma	CD20	Kills B cells with ^{131}I	Non-Hodgkin lymphoma	Bexxar®
-ximabs						
Abciximab	Chimeric IgG1κ Fab	Sp2/0	Glycoprotein IIb/IIIa	Platelet aggregation inhibitor	Cardiovascular disease	Reopro®
Basiliximab	Chimeric IgG1κ	Sp2/0	α chain IL-2 receptor (CD25)	Reduces T cell activation	Prevent organ transplant rejection	Simulect®
Brentuximab vedotin[d]	Chimeric IgG1κ[d]	CHO	CD30	Antimitotic MMAE[d]	ALCL and Hodgkin lymphoma	Adcetris®
Cetuximab	Chimeric IgG1κ	Sp2/0	EGFR[e]	Turns off cell division	Colorectal and head and neck cancers	Erbitux®
Infliximab	Chimeric IgG1κ	Sp2/0	TNF	Inhibits TNF-α	Autoimmune disease (RA, Crohn's)	Remicade®
Rituximab	Chimeric IgG1κ	CHO	CD20	B cell killing	Non-Hodgkin lymphoma	MabThera® Rituxan
-zumabs						
Alemtuzumab	Humanized IgG1κ	CHO	CD52	Eliminates lymphocytes	Chronic lymphocytic leukemia	Campath-1H®
Bevacizumab	Humanized IgG1κ	CHO	VEGF[f]	Angiogenesis inhibitor	Colorectal cancer	Avastin®
Certolizumab pegol[g]	Humanized IgG1κ Fab, pegylated[g]	*E. coli*	TNF	Inhibits TNF-α	Crohn's and RA	Cimzia®
Eculizumab	Humanized IgG2/4κ	NSO	C5[h]	Inhibits cleavage of C5	Paroxymal nocturnal hemoglobinuria	Soliris®
Natalizumab	Humanized IgG4κ	NSO	α-4 integrin[i]	Blocks lymphocyte trafficking	MS and Crohn's	Tysabri®
Omalizumab	Humanized IgG1κ	CHO	IgE	Removes IgE	Chronic asthma	Xolair®
Palivizumab	Humanized IgG1κ	NSO	RSVF[j]	Inhibits virus entering cell	RSV	Synagis®
Ranibizumab	Humanized IgG1κ Fab	*E. coli*	VEGF[f]-A	Angiogenesis inhibitor	Macular degeneration	Lucentis®
Tocilizumab	Humanized IgG1κ	CHO	IL-6R	Blocks IL-6-induced inflammation	RA	Actemra®
Trastuzumab	Humanized IgG1κ	CHO	HER2[k]	Prevents over expression of HER2	Breast cancer	Herceptin®
-(m)umabs						
Adalimumab	Human IgG1κ	CHO	TNF	Inhibits TNF-α	Autoimmune disease (RA, Crohn's)	Humira®
Belimumab	Human IgG1λ	NSO	BLys[l]	Inhibits immuno-stimulant BLys	Systemic lupus erythematosus	Benlysta®

Canakinumab	Human IgG1κ	Sp2/0	IL-1β	Blocks inflammation by IL-1β	CAPS[m]	Ilaris®
Denosumab	Human IgG2κ	CHO	RANK-L	Inhibits activation of osteoclasts by RANK-L	Bone loss	Prolia® Xgeva®
Golimumab	Human IgG1κ	Sp2/0	TNF	Inhibits TNF-α	Autoimmune disease	Simponi®
Ipilimumab	Human IgG1κ	CHO	CTLA-4[n]	Blocks interaction of CTLA-4 with its ligands[o]	Metastatic melanoma	Yervoy®
Ofatumumab	Human IgG1κ	NSO	CD20	B cell killing	Chronic lymphocytic leukemia	Arzerra®
Panitumumab	Human IgG2κ	CHO	EGFR[e]	Turns off cell division	Colorectal cancer	Vectibix®
Ustekinumab	Human IgG1κ	Sp2/0	IL-12, IL-23	Inhibits inflammation by IL-12, IL-23	Psoriasis	Stelara®
Pending						
Pertuzumab	Humanized IgG1κ	CHO	HER2[k]	Inhibits dimerization of HER2 with other HER receptors[p]		
Raxibacumab	Human IgG1κ	NSO	*B. anthracis* protective Ag	Antitoxin activity. In review		

RA rheumatoid arthritis. *CHO* Chinese hamster ovary cells; Sp2/0, BALB/c mouse spleen cells fused with P3 myeloma. Cells do not secrete Ig, are resistant to 8-azaguanine, and are HAT sensitive. NSO, non-Ig secreting, non-L-chain synthesizing. 8-Azaguanine-resistant and HAT-sensitive mouse myeloma cell line

[a]Specificity of mAb

[b]With Yttrium-90 or Indium-111

[c]*ADCC* antibody-dependent cell-mediated cytotoxicity, *CDC* complement-dependent cytotoxicity

[d]Conjugated to the cytotoxic agent monomethyl auristatin E (MMAE)

[e]*EGFR* epidermal growth factor receptor

[f]*VEGF* vascular endothelial growth factor (a subfamily of growth factors; includes VEGF-A)

[g]Attached to PEG (polyethylene glycol)

[h]Complement component 5

[i]α-4 integrin (CD49d)

[j]*RSV* Human respiratory syncytial virus; F (viral protein coat antigen)

[k]*HER2* human epidermal growth factor receptor. Also known as Neu, ErB-2, CD340, or p185

[l]*BLys* B lymphocyte stimulator; B-cell activating factor, BAFF

[m]*CAPS* cryopyrin-associated periodic syndrome; Muckle–Wells syndrome

[n]*CTLA-4* cytotoxic T-lymphocyte antigen 4; CD152

[o]Ligands for CTLA-4—CD80/CD86

[p]Approved by FDA June 2012 for metastatic breast cancer. Trade name, Perjeta®

infusions of anti-immune cell mAbs (again, such as rituximab). It is thought to be a consequence of antibody binding to, and activation of, the cells producing a systemic inflammatory response together with high fever. The reaction, which is similar in some respects to infection, can induce life-threatening pulmonary edema and possibly death. Systemic inflammatory response syndrome affects the whole body and resembles the response seen to sepsis. It may lead to respiratory distress syndrome, renal failure, gastrointestinal bleeding, and dysfunction of the central nervous system.

Although there are many reports of adverse reactions, especially infusion reactions, to many of the mAbs in current use, most information is available on five widely and frequently used antibodies and they will therefore be considered in some detail. Reactions seen to these four mAbs, ranging from mild skin rashes to full-blown anaphylaxis and including infusion reactions, are similar to those seen with the other antibodies.

11.1.3.1 Omalizumab

Omalizumab, a humanized IgG1κ mAb with specificity for human IgE antibodies (Table 11.1), is approved for the treatment of severe allergic asthma in patients 12 years or older. It binds to free, circulating IgE antibodies and membrane-bound IgE molecules on some cells such as B lymphocytes expressing the antibody, but it does not bind to IgE already bound to mast cells, basophils, and dendritic cells. This selectivity of binding results from omalizumab binding to a determinant in the Cε3 region of the free antibody, the same region involved in binding to the FcεRI receptor on the mast cell. Interference with binding due to steric hindrance also occurs when IgE is bound to the receptor, in this case preventing the binding of the mAb to the patient's IgE molecules. Omalizumab has proven extremely efficient in depleting free circulating IgE to almost negligible levels with two interesting consequences. As IgE levels are reduced, the complementary receptors on mast cells, basophils, and dendritic cells fall correspondingly and this has the consequence of rendering the cells less sensitive to allergen stimulation. Secondly, antigen trapping by IgE and subsequent presentation by dendritic cells are markedly reduced or prevented resulting in no further activation of allergen-specific Th2 cells.

Despite the clear efficiency of omalizumab in reducing levels of IgE antibodies, the question of its safety remains paramount. In one assessment of the mAb's safety and tolerability, data from completed clinical studies involving more than 7,500 patients with asthma, rhinitis, and other conditions were reviewed with a focus on hypersensitivity reactions, other immune effects, thrombocytopenia, malignant neoplasia, and parasitic infections. Findings revealed that omalizumab had good safety and tolerability records that were maintained for up to 4 years in one study. The incidence of anaphylaxis to the agent was 0.14%, twice the figure seen in control patients, but based on an estimated exposure of 57,300 patients, the frequency of anaphylaxis was estimated to be at least 0.2 %. Increased risks of malignant neoplasia and thrombocytopenia were not detected. A review undertaken by the U.S. Food and Drug Administration Adverse Event Reporting System of cases of anaphylaxis to omalizumab for the period June 2003 and December 2006 revealed 124 cases, many of whom experienced delayed (>2 h) onset of the reaction or protracted progression of the symptoms. A similar review for the period June 2003 to December 2005 carried out by a joint task force of the major U.S. allergy societies found 41 cases of anaphylaxis in 35 of 39,510 patients given omalizumab, a rate of 0.104 %. These figures show that in 2006, another 83 episodes of anaphylaxis were recorded and this finding, together with the higher incidence of anaphylaxis in the post-marketing period than in the pre-marketing clinical trials, led the Omalizumab Joint Task Force to issue guidelines for the administration of the agent. The Task Force recommended: (1) Prior to administering omalizumab, patients should be assessed for vital signs, asthma control, and lung function; (2) Informed consent should be obtained; (3) Patients should be advised how to recognize anaphylaxis, how to use an epinephrine auto-injector and to

ensure that the injector is always available during the administration of omalizumab; (4) Omalizumab should only be administered in a facility that has both the staff and equipment to treat anaphylaxis; and (5) Patients should be observed for 2 h after each of the first three injections and for 30 min after subsequent injections of the mAb.

11.1.3.2 Cetuximab

Cetuximab, a chimeric mouse-human IgG1κ mAb to the epidermal growth factor receptor (EGFR) used to treat colorectal cancer and squamous cell cancer of the head and neck, has been shown to be associated with anaphylaxis in a most curious way. Natural antibodies, mostly of the IgG class, specific for an α-1,3-linked D-galactose disaccharide, a structure found in many animals but not humans, are found in all individuals as a result, it is thought, of inactivation of the gene for the enzyme α-1,3-galactosyltransferase. Presumably, this resulted in the loss of immune tolerance to the α-D-galactose determinant and the production of antibodies to it. Although the biological role of this antibody remains unclear, it may provide some protection against gastrointestinal bacteria and contribute to the removal of senescent red cells via recognition of cryptic α-D-galactosyl residues exposed in the course of cell aging. Humans of blood group B have a terminal α-1,3-linked-d-galactose on their blood group substances in secretions and on red cells, but the presence of a penultimate α-1,2-linked L-fucose prevents binding to the antibody. By the early 1990s it was known that the anti-D-galactosyl antibodies in humans were potentially capable of interacting with therapeutic recombinant proteins expressing the complementary α-linked determinant and this became a reality just a few years ago when patients receiving cetuximab experienced severe immediate hypersensitivity reactions. The antibodies involved were found to be IgE, specific for α-D-galactose-(1–3)-β-d-galactose and reactive with this disaccharide present on the Fab portion of the chimeric antibody at asparagine 88 of the heavy chains. Most of the patients who reacted already had the IgE antibodies in their serum before administration of cetuximab, but how they became sensitized to the disaccharide in the first place remains uncertain. A possible explanation was suggested by the occurrence in patients given cetuximab of delayed-onset anaphylaxis, angioedema, and/or urticaria 3–6 h after consuming red meat. Following some investigations and questioning of patients in a large area of the U.S. South East, it has been speculated that IgE antibodies to the α-linked D-galactose disaccharide present in the meat may be linked to prior tick bites. Previous results by Van Nunen and colleagues in Sydney who reported an association between reactions to tick bites and allergy to red meats appear to support this suggestion. The mechanism of this association is, as yet, unknown and is itself subject to speculation. An anti-cetuximab IgE ELISA was recently reported with the claim that it could be a valuable test to identify potential cases of anaphylaxis following cetuximab infusion. Cetuximab-reactive IgE antibodies were detected in 24 of 92 (26.1 %) of pretreatment patients and 33 of 117 (28.2 %) healthy blood donors. Hypersensitivity reactions occurred in 14 of the 92 patients (15.2 %) and 8 of these were grade 3–4 reactions. Seven of the eight patients (87.5 %) with severe hypersensitivity reactions had anti-cetuximab IgE antibodies while 14 of 78 (17.9 %) with no signs of hypersensitivity showed the presence of antibodies.

With the greatly expanding administration of biologic agents, it is already clear that infusion reactions occur frequently, and because many patients being treated with mAbs often have few or even no other therapeutic alternatives, successful desensitization to mAbs is likely to be increasingly sought. Claim of a successful desensitization protocol to cetuximab has been published. The patient was premedicated with prednisolone 12 h and 1 h before desensitization and with diphenhydramine 30 min before desensitization. Beginning with an infusion dose of 0.001 mg of cetuximab, doses were doubled every 15 min with each dose tolerated until a total of 64 mg of cetuximab was reached. The appearance of a pruritic cutaneous reaction was managed with diphenhydramine, a 30 min waiting period, and dose and infusion rate reductions. The final dose

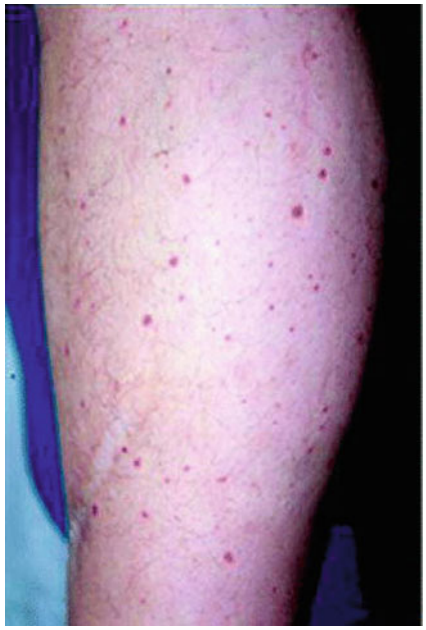

Fig. 11.1 Palpable purpura in a patient with hypersensitivity vasculitis (leukocytoclastic vasculitis), a small vessel vasculitis usually involving post-capillary venules in the dermis. This cutaneous manifestation of vasculitis occurs occasionally following treatment with mAbs and some other biologic agents (Photograph of Dr. John Stone) (Reproduced with permission from Weyand CM, Goronzy J, in Klippel JH, Stone JH, Crofford LeJ, White PH, editors. Primer on the Rheumatic Diseases, 13th ed. New York: Springer, 2008)

of cetuximab (325 mg) was tolerated giving a cumulative dose of 844 mg. When challenged with cetuximab 1 week later, the patient tolerated the dose without difficulty. This was expected since the half-life of cetuximab is approximately 4 days. It was predicted that the protocol might be useful to many patients.

11.1.3.3 Infliximab

Like cetuximab, infliximab is a mouse-human chimeric IgG1κ mAb. With specificity for TNF, the antibody is used to treat autoimmune diseases such as Crohn's disease and rheumatoid arthritis (Table 11.1) and there are a few reports of it inducing rapid recovery of lesions in several cases of toxic epidermal necrolysis (Sect. 3.6.3.7). A variety of reactions, both systemic and cutaneous, have been reported following administration of infliximab. These include maculopapular rashes, urticaria, psoriasis, flare-up of atopic dermatitis, leukocytoclastic vasculitis (Fig. 11.1), serum sickness, and anaphylaxis. In a large center study of 165 consecutive patients who received 479 infliximab infusions, the overall incidence of infusion reactions to the mAb was 6.1 % with 9.7 % of the patients affected. Mild, moderate, and severe reactions occurred in 3.1, 1.2, and 1 % of infliximab infusions, respectively. Serum tryptase levels suggested that acute reactions in 11 of 14 patients were not type I hypersensitivity reactions. Delayed reactions were rare, resulting from only 0.6 % of infusions. An examination of incidences of systemic and delayed reactions to infliximab in children as well as adults with Crohn's disease revealed that 14 % of 86 patients experienced severe systemic reactions from a total of 304 infusions. A significant difference between the results for adults and children was noted—severe systemic reactions were seen in 11 of 52 adults (21.2 %) and in only one of 34 (2.9 %) children. These reactions were characterized by hypotension, mucosal irritability, and laryngospasm requiring epinephrine, antihistamines, and/or corticosteroids. Delayed reactions, which manifested as arthralgia, fever, and myalgia requiring corticosteroids were seen in eight adults (9.3 %); no delayed reactions occurred in children. An examination of the extent of, and reasons for, discontinuation of infliximab treatment in 84 patients with established rheumatoid arthritis revealed that 28 (33 %) discontinued the therapy. The main reason for discontinuation was an adverse reaction in 16 of the 84 patients (19 %). In this group, 9 of the 16 patients (10.7 %) experienced an immediate hypersensitivity reaction. There are many reports of immediate reactions, in particular anaphylaxis, to infliximab. From FDA and other sources, over 650 anaphylactic reactions to the mAb at an incidence of just under 0.9 % occurred in the period 1999–2012. Most reactions (nearly 70 %) occur during the first month of therapy; females account for approximately 60 % of reactors and children under 10 years of age for 7.5 % of reactors.

Cutaneous reactions may also be provoked by infliximab. Three patients with rheumatoid arthritis, none of whom had a personal or family history of psoriasis, developed what was described as psoriasiform skin lesions 6–9 months after the initiation of infliximab therapy. Two of the

patients developed palmoplantar pustular lesions and scaly plaques on the extremities, while the third patient had erythematous plaques with silvery white scales on the scalp. Other rare adverse reactions to infliximab that may have an immunological basis include demyelinating polyneuropathies, peripheral neuropathy, drug-induced lupus, and hepatitis. A combination of the latter two conditions has been reported with the use of infliximab for psoriasis.

Successful desensitizations have been reported in adult and child patients who experienced an anaphylactic/anaphylactoid reaction to infliximab. Based on the adult patient's weight of 70 kg and the standard dose of 5 mg/kg, a total infliximab infusion of 353 mg divided into 11 increments was given IV every 15 min over a 4 h period with a starting dose of 3 μg and a final dose of 160 mg. For a 10-year-old male child, the schedule was the same except for the lower dosages (total of 208 mg), increments ranging from 2 μg to 80 mg and a final dose of 80 mg. For both patients, IV infliximab was tolerated without incident and each experienced clinical improvement over the subsequent 2 months of treatment.

11.1.3.4 Adalimumab

Like infliximab, adalimumab is a mAb targeted at TNF and used for Crohn's disease and rheumatoid arthritis, but, unlike infliximab, it is a fully human mAb of the IgG1κ class and can be administered subcutaneously. Being a possible substitute for infliximab in patients intolerant to that mAb, in 2004 adalimumab was examined for safety and efficacy in seven patients who had experienced immediate or delayed hypersensitivity reactions to infliximab and one patient with infliximab-induced lupus. Adalimumab proved to be well tolerated leading to the conclusion that it might prove to be a safe and effective substitute for patients allergic or otherwise intolerant to infliximab, In subsequent years, a range of different apparent hypersensitivity reactions induced by adalimumab have become apparent. These include psoriasis, exacerbation of palmoplantaris pustulosa psoriasis, asthma, bronchospasm, autoimmune hepatitis, and a number of different skin reactions, some severe. Like infliximab, adalimumab has been reported to elicit psoriasiform plaques on elbows, arms, and thighs together with palmoplantar pustular lesions. More severe cutaneous reactions to the mAb also occur such as erythema multiforme-like skin reaction with papulopustular exanthema at the injection site and on the palms and soles followed by skin desquamation and at least two cases of Stevens–Johnson syndrome. Systemic reactions to adalimumab have been reported. In one case, a patient with spondylarthritis treated with the mAb experienced two reactions consisting of generalized itching, angioedema of the lips, dizziness, and visual disturbances. Skin prick and intradermal tests with adalimumab produced strong immediate positive reactions, but serum IgE antibodies were not detected using a specially prepared adalimumab Phadia solid phase.

11.1.3.5 Rituximab

Rituximab, like cetuximab and infliximab, is a *-ximab* chimeric IgG1κ mAb (Table 11.1). It contains murine light and heavy chain variable region sequences and human constant region sequences. Administered for non-Hodgkin lymphoma, rituximab is targeted to CD20 (human B lymphocyte-restricted differentiation antigen Bp35), a B lymphocyte antigen involved in the development and differentiation of B cells into plasma cells. CD 20 is found on B cell lymphomas, B cell chronic lymphocytic leukemia, hairy cell leukemia, and melanoma cancer stem cells and is expressed on more than 90 % of B cell non-Hodgkin lymphomas but not on normal plasma cells, hematopoietic stem cells, or other normal tissues. In 1997 rituximab was the first mAb approved specifically for cancer therapy. In the early years following its release, a relationship between cytokine release syndrome in patients and high lymphocyte counts was observed after treatment with the mAb. Patients with lymphocyte counts greater than 50×10^9/L experienced a severe cytokine release syndrome shown by peaks in release of TNF and IL-6 90 min after infusion and accompanied by fever, chills, nausea, vomiting, hypotension, dyspnea, an increase in liver enzymes, and prolongation of the prothrombin time. When used to treat B cell

cancers, the frequency and severity of first-dose reactions to rituximab were shown to be dependent on the initial number of circulating tumor cells—patients with counts exceeding 50×10^9/L experienced more adverse reactions than patients with lesser numbers of peripheral tumor cells.

Recently published results of a survey of rituximab hypersensitivity reactions in patients at Massachusetts General Hospital between 2006 and 2010 showed that immediate hypersensitivity reactions to rituximab occurred in 8.8 % (79 of 901) of patients treated with the mAb and premedications. Approximately three-quarters of the patients developed symptoms after the first infusion and 46 % of moderate or severe reactions occurred on subsequent infusions. Severity of reactions correlated with the risk of a recurrent adverse response—all patients with severe, and 56 % of those with moderate reactions, but only 36 % of patients with mild reactions, experienced a recurrence. Interestingly, there was an increased risk of a moderate or severe hypersensitivity reaction in those with advanced disease. Waldenstrom's macroglobulinemia accounted for 10 % of all reactions while representing only 1 % of patients treated with rituximab. In another retrospective analysis of reactions occurring over a 2-year period following infusion of rituximab in the treatment of multiple sclerosis, 25.7 % of patients had mostly mild to moderate reactions, most often during the first infusion. Most patients completed the infusion and went on to subsequent infusions without reactions. In common with a number of other surveys on rituximab use, it was concluded that infusion-related reactions to the therapeutic agent in patients with multiple sclerosis are common, premedication with drugs including corticosteroids dramatically reduces the incidence of reactions, and reactions that do occur can be effectively managed by treatment with H_1 and H_2 antihistamines and infusion rate adjustments.

As well as infusion-related reactions and reactions related to the number of circulating target cells, a number of post-infusion hypersensitivity or hypersensitivity-like reactions occur to rituximab. These reactions include serum sickness, vasculitis, various cutaneous manifestations, interstitial pneumonitis, and acute respiratory distress syndrome. Respiratory events such as cough, dyspnea, and bronchospasm are fairly common adverse reactions, but serious reactions such as fatal pulmonary fibrosis also occur. A review of 62 cases of rituximab-induced severe respiratory reactions revealed that 74 % suffered from interstitial pneumonitis and other respiratory problems including pulmonary fibrosis, bronchiolitis obliterans, organizing pneumonia, hypersensitivity pneumonia, and acute respiratory distress syndrome. Most patients were elderly but two pediatric patients, both with refractory nephrotic syndrome, were affected. Such findings have led to the suggestion that rituximab should never be administered to patients suffering from lung diseases such as pneumonia, pleural effusion, and atelectasis. Other mAbs including infliximab, gemtuzumab, OKT3, and a mAb-ozogamacin conjugate have also been implicated in cases of acute respiratory distress syndrome which is believed to be mediated by pro-inflammatory cytokines. Cutaneous side effects of rituximab are frequent, generally occur from 1 to 13 weeks after exposure, and are not usually serious but Stevens–Johnson syndrome, toxic epidermal necrolysis, lichenoid dermatitis, and vesiculobullous dermatitis have been described.

11.1.3.6 The Next Generation of Monoclonal Antibodies

Until now, FDA-approved mAbs (Table 11.1) are usually full-length antibodies, making them large molecular weight (~150 kDa) therapeutic agents with sometimes poor tissue penetration, especially for solid tumors. As understanding of the biological mechanisms in different diseases increases and genetic engineering technology advances, attention is turning to improving the performance and efficiency of mAbs in terms of increased selectivity, improved pharmacokinetics, higher binding affinities, more efficient cytotoxicity, better tissue penetration, and increased half-life in serum. More than 50 % of mAbs in phase I clinical trials and about 40 % of those in phases II and III are now modified antibodies such as antibody–drug conjugates, bispecific antibodies, antibodies with modified Fc

functions, and antibody fragments/domains. Employment of two mAbs may sometimes produce a better therapeutic outcome than one antibody alone, for example, the combination of cetuximab with specificity for EGFR and bevacizumab which recognizes VEGF, in the treatment of metastatic colorectal cancer. A more logical and efficient alternative to the use of more than one mAb is the creation of an antibody with specificities for two different targets, that is, bispecific antibodies. By simultaneously binding to two different complementary sites on a cell, bispecific antibodies may enhance binding selectivity, avidity, and tissue distribution, expand the mAb's disease indications, and increase antibody load on target cells. Nonspecific toxicity of chemotherapy is a major limitation of much of today's drug therapy for cancers (see chapter 13). Antibodies conjugated to carefully selected drugs or toxins delivered to specific tumor sites have the potential to reduce systemic toxicity and there is little doubt that such mAb drug conjugates will be increasingly developed and applied in future mAb immunotherapies. One recent imaginative approach in applying antibody–drug conjugates for cancer therapy is based on the occurrence of angiogenesis in virtually all types of aggressive cancers but the rarity of the process in healthy adults. Early experiments have demonstrated that strong antitumor activity in vivo can be achieved with vascular targeting of an antibody–drug conjugate that does not require antibody internalization. Fc engineered mAbs can be utilized for improved antibody-dependent cellular and complement-dependent cytotoxicities via their Fc regions and antibody fragments and single domain antibodies offer the opportunity to retain or enhance the binding properties of full-length antibodies by varying their size, valency, and pharmacokinetic profiles. Another potential improvement offered by antibody fragments is improved tissue penetration although this may be offset by a shorter serum half-life. Approaches already employed to overcome this include the chemical addition of polyethylene glycol (PEG) or so-called PEGylation to increase the size of fragments. This strategy was applied to certolizumab, an anti-TNF Fab fragment.

What effects these developments will have on the incidences and nature of adverse, and in particular allergic, reactions to therapeutic mAbs remains to be seen, but it is certain that the number and variety of genetically engineered mAbs and modified mAbs will greatly increase in the immediate future. We might expect that many reactions already encountered with the existing approved antibodies will still be seen, but we should not be surprised if some new, unanticipated adverse reactions emerge as newer generation agents showing some physical, chemical, and biological differences become established and increasingly used. However, as summarized above, the carefully planned strategies already being applied to modify and thereby improve the performance and tolerability of next generation mAbs (e.g., smaller size and intrabodies) may also carry with them a decreased capacity to provoke adverse responses in patients. In any case, the continuing elucidation of cellular pathways in an expanding range of diseases coupled with a deeper understanding of the myriad immunological and inflammatory processes and the advancing bioengineering expertise should give hope that such intellectual and technical ingenuity could also be applied to minimizing the detrimental effects of these precisely engineered therapeutic agents. Efforts expended to make mAbs more immunologically acceptable to humans have been impressive; equal efforts directed at minimizing adverse reactions might prove just as successful.

11.2 Etanercept

Like the mAbs infliximab, certolizumab, adalimumab, and golimumab, etanercept is specific for, and binds to, TNF, thus making it useful for the treatment of some autoimmune diseases including rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, plaque psoriasis, and Crohn's disease. TNF receptors are of two types, those on nucleated cells and soluble receptors that circulate and deactivate the cytokine. Etanercept is a recombinant, engineered, fully human dimeric fusion protein of molecular weight 150 kDa, made up of

the extracellular ligand-binding portion of human 75 kDa TNF receptor (TNFR) linked to an Fc portion of human IgG1. The Fc portion contains the CH2 and CH3 domains and the hinge region but not the CH1 domain. In this form, the protein acts as a "decoy" receptor for TNF, mimicking the natural soluble receptor and improving on it by possessing a longer half-life (115 h; compare infliximab half-life 210 h). Both infliximab and etanercept are sometimes used for patients with rheumatoid arthritis when other disease-modifying antirheumatic drugs have failed. The most common side effects reported for etanercept include mild reactions at the injection site, infections (mainly upper respiratory), and sinusitis. U.S. FDA data on etanercept adverse events lists, in order of frequency, infections, followed by dermatologic, neurologic, musculoskeletal, pulmonary, cardiac, and vascular effects. Both etanercept and infliximab have been associated with cutaneous vasculitis in which symptoms coincided with their administration and symptom resolution coincided with treatment withdrawal. Lesions associated with etanercept-induced autoimmune skin reactions include purpuric papules and erythematous papules and nodules on the trunk and extremities. In one reported case of cutaneous vasculitis associated with both etanercept and infliximab, vasculitis provoked by etanercept in a patient with Crohn's disease was found to worsen significantly after therapy was switched to infliximab.

11.3 Interferons

Interferons (IFNs) are an important class of broad spectrum antiviral cytokines found in higher animals, reptiles, fish, and birds. IFNs make up a large, still growing list of proteins, seven of which occur in humans. These are further divided into three classes types I, II, and III. Two important type I IFNs in human are IFNα and IFNγ. There are at least 14 different alpha IFNs. These are produced by leukocytes and dendritic cells and are part of an innate immune response with antiviral action. IFNβ, produced by fibroblasts and probably many other cells, is used in the treatment of multiple sclerosis. It inhibits viral replication and is the product of a single gene. Two synthetic IFNs, pegylated IFNα-2a and pegylated IFNα-2b, have found application as antiviral agents in the treatment of hepatitis C virus. IFNα, now usually given in pegylated form, in combination with ribavirin (1-β-D-ribofuranosyl-1*H*-1,2,4,-triazole-3-carboxyamide) is the mainstay of treatment of hepatitis C infection. Pegylation enhances the half-life of the IFN by covalent attachment of polyethylene glycol (PEG) polymer chains to the molecule, reducing both antigenicity and immunogenicity. Adverse reactions to IFNα include flu-like symptoms, localized inflammation, and a wide range of cutaneous reactions including maculopapular rash, pruritus, urticaria, angioedema, pemphigus, vasculitis (and systemic), fixed drug eruption, and lichen planus. Autoimmune disorders thrombocytopenia and hemolytic anema also occur. Cytokine release syndrome probably accounts for many reactions with flu-like symptoms. Recombinant IFNβ has been implicated in anaphylactic shock, indicating that IgE-mediated reactions are probably involved in a few immediate reactions. Of 51 patients given pegylated IFNα-2b-ribavirin, 10 were found to have experienced serious adverse drug reactions after 1 month and adverse reactions were the main reason for discontinuing the therapy in eight patients (15.7 %). The most common reaction to this therapeutic agent is localized skin lesions at the injection site, but vesicle erythematous eruptions and autosensitization dermatitis away from the injection site have been described.

11.4 Interleukin 2

The cytokine interleukin 2 (IL-2) is necessary for T cell growth, proliferation, and differentiation. An FDA-approved recombinant human IL-2 is used clinically as Proleukin® (Prometheus Laboratories) for the treatment of metastatic melanoma and renal cell carcinoma. Erythema during IL-2 immunotherapy is common and was well described in a French study nearly 20 years ago with a report on generalized erythema followed by desquamation in 12 patients treated with the cytokine for renal cancer. Urticaria in eight renal cell cancer patients after the end of IL-2 therapy has also been reported. Skin tests with IL-2 on

two of the patients proved negative. Cutaneous side effects in metastatic melanoma patients treated with IL-2 were observed in 53 of 78 treatment cycles of 25 patients (72 %); 53 were mild reactions with a burning pruriginous erythema, and three were severe with urticaria, necrotic lesions, and blisters. Regression proved constant without sequelae. Other observed cutaneous reactions to IL-2 include injection site reactions, exfoliative dermatitis, angioedema, reactivation of eczema, vasculitis, pemphigus, and exacerbation of psoriasis. There appear to be no reports of anaphylaxis following IL-2 immunotherapy.

11.5 Denileukin Diftitox and Aflibercept

Diphtheria toxin is a single polypeptide chain of 535 amino acids. For use as a targeted toxin, it has been modified by deleting the 147 amino acid residue receptor-(cell-) binding domain to produce a protein of 388 amino acids commonly referred to as DT_{388} or DAB_{389}. This remaining protein consists of the adenosine diphosphate (ADP)-ribosyltransferase and membrane translocating domains of native diphtheria toxin. Replacing the receptor-binding domain of the native toxin by the sequences encoding the IL-2 gene produced the recombinant fusion toxin designated DAB_{389}IL-2 or denileukin diftitox (Ontak®, Eisai) which was approved by the FDA in 1999 for the treatment of cutaneous T cell lymphoma. Bound to the IL-2 receptor, the fusion toxin undergoes endocytosis and is proteolytically cleaved liberating the modified toxin and causing ADP-ribosyltransferase-mediated inhibition of protein synthesis. In phase I and III studies on DAB_{389}IL-2, infusion-related acute hypersensitivity reactions occurred in 70 % of patients and vascular leakage occurred in 27 % forcing 29 % of patients to discontinue therapy. There were no correlations between these reactions and the dose or the half-life of the fusion toxin. Since only the antihistamine diphenhydramine and acetaminophen were used for premedication in these studies, the steroids prednisone and dexamethasone were examined to see if they improved tolerability of DAB_{389}IL-2. Results showed that the incidence of acute infusion reactions decreased significantly with only three patients experiencing reactions and only two patients developing vascular leakage. Overall, a significant improvement of 60 % occurred when premedication with steroids was employed. Cutaneous reactions to denileukin diftitox include injection site reaction, erythema, and pruritus, and there has been one fatal case of toxic epidermal necrolysis.

Another recombinant fusion protein, *aflibercept* (Zaltrap®), used to treat colorectal cancer and macular degeneration, is a chimera of the Fc piece of IgG1 and the extracellular ligand-binding domains of human vascular endothelial growth factor receptors VEGFR1 and VEGFR2. Adverse/hypersensitivity reactions to the drug include cytopenias, hemorrhage, thromboembolism, GI perforation, acral erythema and stomatitis.

11.6 Anakinra

Interleukin-1 (IL-1) is a cytokine produced in response to inflammatory stimuli in conditions such as rhinitis, rheumatoid arthritis, and other immunological reactions. The IL-1 receptor (IL-1R) exists in both membrane-bound and soluble forms and is expressed on many organs and tissues. Anakinra, a specific receptor antagonist for IL-1, is a 153 amino acid non-glycosylated, molecular weight 17.258 kDa recombinant protein prepared in *Escherichia coli*. It differs from natural IL-1R by the addition of a single methionine added to the amino terminal end. The recombinant receptor antagonist competes with IL-1, blocking its access to its complementary receptor, thus making it a useful agent in the treatment of some inflammatory conditions such as rheumatoid arthritis where it acts as a biological response modifier rather than a disease-modifying antirheumatic drug. The protein has a half-life of 4–6 h and peak plasma concentrations occur 3–7 h after subcutaneous injection. In an examination of the safety profile of anakinra, over 1,300 patients were initially studied in a double blind trial comparing the drug (100 mg/day) with placebo before proceeding to open-label treatment for up to 3 years. All adverse events were similar in the anakinra and placebo groups and for each of the years of anakinra treatment. Injection

site reactions were the most frequent adverse event (122 events/100 patient years) and, overall, it was concluded that anakinra is safe and well tolerated for up to 3 years of continuous use. Cutaneous reactions are usually at the injection site and usually well tolerated. Skin biopsy specimens from rheumatoid arthritis patients treated with anakinra and with well-defined erythema and edema at the injection sites showed marked dermal edema, an increased number of mast cells, and a lichenoid infiltrate of mainly lymphocytes together with eosinophils and CD68 macrophages. In some cases, cutaneous reactions were associated with systemic involvement. The observed skin reactions were said to resemble reactions seen in patients receiving chemotherapy and colony-stimulating factors. A cutaneous reaction in one patient was shown to be mediated by specific IgE antibodies. After several injections of anakinra, an adult female patient began to experience erythema and pruritus at the injection site within 15 min after each injection. No systemic symptoms occurred, but 1 day later swelling, erythema, and pruritus were apparent and these persisted for a further day or two. Prick and intradermal tests together with an ELISA for specific IgE antibodies to anakinra were undertaken. Prick tests with concentrations of 500 and 2,500 μg/ml were negative, but intradermal testing with 125 μg proved positive. The reaction was still positive after 48 h. Five normal control subjects had no reaction to the same dose of the drug. The specific IgE test was also positive and this was confirmed by inhibition experiments with free anakinra.

11.7 Anti-thymocyte Globulin

Indicated and approved for the management of allograft rejection in renal transplant patients, anti-thymocyte globulin preparations are purified immune globulins (primarily IgG) from horses or rabbits immunized with human thymus lymphocytes. The resultant globulin preparations contain cytotoxic antibodies to human T lymphocytes which function as an immunosuppressive agent. As well as its use for the treatment of renal transplant rejection, anti-thymocyte globulin may be administered as an adjunct to other immunosuppressive therapy to delay rejection. Two preparations are available, Thymoglobulin® (Genzyme), obtained by immunizing rabbits, and Atgam® (Pfizer), from hyperimmune horse serum. These two preparations are contraindicated in patients with a history of allergy and anaphylaxis to rabbits or horses, respectively. Serious immune-mediated reactions have been reported, including anaphylaxis, severe cytokine release syndrome, and severe acute infusion-associated reactions. The last named may occur as early as the first or second infusion and serum sickness with fever, rash, arthralgia, and myalgia may appear 5–15 days after the initiation of therapy. As a precaution against the possibility of anaphylaxis, it is recommended that before the first infusion, patients should be tested intradermally with the diluted globulin preparation (for example, with ~5 ug of horse globulins). The most frequently seen adverse reactions, that is, seen in more than 25 % of patients, include fever, chills, headache, nausea, diarrhea, abdominal pain, peripheral edema, hypertension, thrombocytopenia, infection, dyspnea, and tachycardia. Cutaneous reactions seen include urticaria, morbilliform eruptions, and acral erythematous eruptions preceding rash. Discontinuation of treatment with anti-thymocyte globulin is recommended upon the appearance of systemic reactions such as anaphylaxis, generalized rash, tachycardia, dyspnea, hypotension, severe thrombocytopenia, or severe leukopenia.

11.8 Epoetins

Human erythropoietin (or erthropoetin) (EPO), also called hematopoietin (or hemopoietin), is a glycoprotein hormone of 165 amino acids MW 34 kDa that controls erythropoiesis (red blood cell production). Recombinant human EPO, introduced in 1986, is available as epoetins alfa, beta, delta, and omega each differing from the endogenous hormone, and from each other, by the individual sugar and sialic acid residues present. Epoetins are administered for renal and non-renal anemias and, despite being given to a large number of patients over a period of 25 years, the preparations have proved poorly immunogenic and few side effects have been observed. In 2001 the FDA and European

Medicines Agency approved a new epoetin, darbepoetin alfa, for treatment of anemia due to renal failure and in patients undergoing immunotherapy. Darbepoetin alfa is a recombinant epoetin molecule containing an extra two *N*-linked oligosaccharide chains introduced to give greater stability and thus allow less frequent administrations. In recent years, there appears to have been an increase in the number of patients developing neutralizing anti-EPO antibodies during therapy and there are now in excess of 250 known cases of pure red cell aplasia. There are also reports of anaphylaxis, rashes, urticaria, and angioedema to the recombinant hormone and injection site reactions are well known. Cutaneous reactions at the sites of former subcutaneous injections of epoetins following the IV injection of different epoetins proved to be the signs of an allergic skin and systemic reaction in a patient with pure red cell aplasia and anti-EPO antibodies. After switching the patient from epoetin alfa to first epoetin beta and then darbepoetin alfa, a systemic anaphylactic/anaphylactoid response occurred and anti-EPO antibodies cross-reactive with epoetin beta and darbepoetin alfa were detected in the patient's serum. This case illustrates that continuation of epoetin therapy in patients with anti-EPO antibodies may carry the risk of a serious systemic (anaphylactic or anaphylactoid) reaction and that skin reactions at the injection site may be the first sign of sensitization which precedes the development of anemia. In three other rare cases, anaphylaxis and serum IgE antibodies, and a generalized eczematous reaction to recombinant EPO, were reported in the presence of negative skin tests to the glycoprotein and acute exanthematous pustulosis was diagnosed after epoetin alfa was replaced by darbepoetin alfa. In an unexpected EPO allergy "false-alarm," the nonionic surface active agent polysorbate 80, and not Eprex® (Janssen) containing epoetin alfa, was shown to be responsible for a case involving generalized pruritus, erythema, and angioedema.

11.9 Human Insulin

Adverse reactions to insulin were not uncommon in the past when the administered preparations were from bovine and porcine sources. The introduction of human recombinant insulin reduced the incidence of reactions, but allergic reactions are still occasionally seen and insulin allergy is now reported to be less than 1 % of diabetic patients treated with insulin. As early as 1982, IgE antibodies to human recombinant insulin that cross-reacted with bovine and porcine insulins were demonstrated in two diabetic patients previously untreated with insulin. Although these patients did not develop any of the clinical manifestations of insulin allergy, large local reactions in association with IgE to human insulin were later seen in a patient within 12 days of insulin initiation therapy with the human recombinant product. The patient showed similar cutaneous reactivity with bovine and porcine insulins despite never having received those preparations, and the insulin-reactive IgE antibodies cross-reacted in vitro with the heterologous proteins. This strongly indicated that common, or markedly similar, antigenic determinants are present on the insulins from all three species. Subsequent experience has confirmed these findings. Skin testing as well as IgE antibody measurements have clearly demonstrated immediate type I reactions to recombinant human insulin and to bovine and porcine insulins in patients never previously given the heterologous insulins. In the investigation of an anaphylactic reaction to human recombinant insulin, employment of skin testing and the Novo Insulin Allergy Kit (Novo Nordisk A/S, Bagsvaerd, Denmark) showed positive intracutaneous tests to 1–100 dilutions of human and porcine insulins and to the genetically engineered recombinant insulin analog, insulin lispro. Once again, the patient had never been treated with porcine insulin in the past. A number of different models have been applied in attempts to desensitize patients allergic to recombinant human insulin. In one successful attempt, insulin lispro was delivered as a continuous infusion via an insulin pump. The delivery and dosage schedule was: 0.7 IU/h for 2–8 h; 0.3 IU/h for 8–13 h; 0.6 IU/h for 13–18 h; 0.8 IU/h for 18–21 h; and 0.6 IU/h for 21–22 h, plus an additional bolus of 6 IU before breakfast, 5 IU before lunch, and 6 IU before dinner. Following this procedure, the allergic reaction did not reoccur. Although the patient remained clinically asymptomatic, the skin prick test to insulin remained positive 3 months later.

Summary

- MAbs were first produced by mouse hybridoma cells prepared by fusing spleen cells from an immunized mouse with mouse myeloma cells. The hybridoma cells retain the capacity to make specific antibody while the myeloma cells impart the capacity of the cells to grow indefinitely in culture, continuously secreting antibody.
- Because mouse antigens rapidly induce an immune response in humans, methods have been developed to humanize mAbs. One approach involves the production of chimeric antibodies. Other methods now produce fully humanized antibodies.
- MAbs of murine origin are designated by the stem *-omab*; chimeric antibodies in which the variable region is spliced into a human constant region are given the *-ximab* stem; humanized antibodies with the murine hypervariable regions spliced into a human antibody have the *-zumab* stem and antibodies with a complete human sequence are given the *-mumab* or *-umab* suffix.
- Currently, ~28–30 mAbs are approved, or under consideration for approval, as specific therapies in the USA or European Union, although about 350 new mAbs for therapeutic application in humans are in the commercial pipeline.
- So far, the number of target antigens for the mAbs is surprisingly small with more than one of the approved antibodies specific for TNF, HER2, CD20, EGFR, or VEGF. Other specificities include EpCAM, glycoprotein IIb/IIIa, CD30, CD52, C5, α-4 integrin, IgE, IL-6R, BLys, IL-1β, and RANK-L.
- Initial infusion reaction to some mAbs, for example, rituximab, may provoke tumor lysis syndrome, cytokine release syndrome, and systemic inflammatory response syndrome.
- Omalizumab, a humanized IgG1κ mAb with specificity for human IgE antibodies, is approved for the treatment of severe allergic asthma. It binds to free, circulating IgE antibodies and membrane-bound IgE molecules on some cells such as B lymphocytes expressing the antibody, but it does not bind to IgE already bound to mast cells. The incidence of anaphylaxis to the mAb is about 0.2 %.
- Some patients receiving cetuximab experienced severe immediate hypersensitivity reactions. The antibodies involved were found to be IgE, specific for α-D-galactose-(1–3)-β-d-galactose and reactive with this disaccharide present on the Fab portion of the chimeric antibody at asparagine 88 of the heavy chains. Some cases of anaphylaxis to the mAb appear to be associated with tick bites and consumption of red meat.
- Systemic and cutaneous reactions have been reported following administration of infliximab. These include anaphylaxis, serum sickness, maculopapular rashes, urticaria, psoriasis, flare-up of atopic dermatitis, and leukocytoclastic vasculitis. The overall incidence of infusion reactions in one study was 6.1 %. Mild, moderate, and severe reactions occurred in 3.1, 1.2, and 1 % of infliximab infusions, respectively.
- Patients with lymphocyte counts greater than 50×10^9/L experienced a severe cytokine release syndrome shown by peaks in release of TNF and IL-6 90 min after infusion with rituximab.
- A number of post-infusion hypersensitivity or hypersensitivity-like reactions occur to rituximab. These reactions include serum sickness, vasculitis, various cutaneous manifestations, interstitial pneumonitis, and acute respiratory distress syndrome.
- As genetic engineering technology advances, attention is turning to improving the performance and efficiency of mAbs in terms of increased selectivity, improved pharmacokinetics, higher binding affinities, more efficient cytotoxicity, better tissue penetration, and increased half-life in serum.
- Etanercept is a recombinant, engineered, fully human dimeric fusion protein of molecular weight 150 kDa made up of the extracellular ligand-binding portion of human 75 kDa TNF receptor linked to an Fc portion of human IgG1. U.S. FDA data on etanercept adverse events lists, in order of frequency, infections, followed by dermatologic, neurologic, musculoskeletal, pulmonary, cardiac, and vascular effects.
- Two synthetic IFNs, pegylated IFNα-2a and pegylated IFNα-2b, have found application as antiviral agents in the treatment of hepatitis C virus.

- Adverse reactions to IFNα include 'flu'-like symptoms, a wide range of cutaneous reactions, autoimmune thrombocytopenia, and hemolytic anemia.
- Reactions to IL-2 immunotherapy include erythema, urticaria, and a variety of other cutaneous reactions. There appears to be no report of anaphylaxis.
- Replacing the receptor-binding domain of the diphtheria toxin by the sequences encoding the IL-2 gene produced the recombinant fusion toxin designated DAB_{389}IL-2 or denileukin diftitox. Cutaneous reactions to denileukin diftitox include injection site reaction, erythema, and pruritus. There has been one fatal case of toxic epidermal necrolysis.
- Aflibercept, used to treat metastatic colorectal cancer and wet macular degeneration, is a fusion protein of the Fc piece of IgG1 and the extracellular ligand-binding domains of human vascular endothelial growth factor receptors VEGFR1 and VEGFR2. It acts as a soluble decoy VEGF receptor and angiogenesis inhibitor. Common adverse reactions include cytopenias, hemorrhage, proteinuria, hypertension, acral erythema, hyperpigmentation, and stomatitis.
- Anakinra, a specific receptor antagonist for IL-1, is a 153 amino acid non-glycosylated, molecular weight 17.258 kDa recombinant protein prepared in *E. coli*. Cutaneous reactions following anakinra medication are usually at the injection site and usually well tolerated. In some cases, cutaneous reactions are associated with systemic involvement.
- Serious immune-mediated reactions to antithymocyte globulin include anaphylaxis, severe cytokine release syndrome, and severe acute infusion-associated reactions. Serum sickness with fever, rash, arthralgia, and myalgia may appear 5–15 days after the initiation of therapy. Cutaneous reactions seen include urticaria, morbilliform eruptions, and acral erythematous eruptions preceding rash.
- Reactions to epoetins are rare, but anaphylaxis to the recombinant forms of the hormone EPO have been reported, one in particular after injection site reactions in a patient with pure red cell aplasia.
- Allergic reactions to recombinant human insulin with cross-reactivity to bovine and porcine insulins occasionally occur.

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