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REVIEW ARTICLE

Chimeric Fusion Proteins Used for Therapy: Indications, Mechanisms, and Safety

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Abstract Chimeric fusion proteins, produced by genetic engineering, are currently made up of effector peptides, for example, a ligand-binding portion of a cytokine or growth factor, extracellular domains of lymphocyte antigens, or a toxin linked to a suitable fusion partner. This review covers eight fusion proteins that have received regulatory approval for human therapy: etanercept, belatacept, abatacept, alefacept, rilonacept, romiplostim, aflibercept, and denileukin-diftitox. Important requirements for an effective fusion protein are effective targeting and binding, cytotoxicity, and a stable molecule with an extended half-life. The Fc region of human IgG1 is generally chosen as the fusion partner for the effector molecule(s) because it extends the fusion protein half-life by recycling via the salvage neonatal FcRn receptor and protects the molecule from lysosomal degradation. Each of the fusion proteins has IgG1 Fc as a fusion partner except denileukin-diftitox, which employs a modified diphtheria toxin effector peptide linked to interleukin-2. For six of the Fc fusion proteins, the effector peptide(s) is linked to the N-terminus of the Fc piece but for the thrombopoietin-mimetic romiplostim, linkage is through the C-terminus. Although some clear type I and IV hypersensitivities are known to be induced by fusion protein therapy, the pathomechanisms underlying many adverse hematologic, respiratory, renal, and cutaneous events have generally not been investigated. Assessment of immunogenicity risk is important because a number of immune-based, or influenced, adverse reactions such as anaphylaxis, cutaneous manifestations, infusion, and injection-site reactions, and cytokine release syndrome can occur. Features of many reactions, some autoimmune in nature, suggest type II, III, or IV hypersensitivities. Clinical findings with the anti-arthritis anti-psoriasis biologic etanercept provide the largest body of current knowledge of fusion protein-induced adverse events. For most fusion proteins, little information is available on appropriate diagnostic and desensitization procedures for hypersensitivity and other adverse responses, although skin test concentrations and some successful desensitization protocols have been published for etanercept.

Key Points

The salvage neonatal FcRn receptor has been exploited to prepare Fc fusion proteins as an efficacious and safe form of biologics therapy.

Effector fusion partners used to date include cytotoxic T lymphocyte-associated antigen 4, lymphocyte function-associated antigen 3, receptors for tumor necrosis factor, interleukin-1, vascular endothelial growth factor, a thrombopoietin-mimetic peptide, and a diphtheria toxin-interleukin-2 fusion protein.

Although Fc fusion proteins are generally safe, adverse events include infusion and injection-site reactions, some autoimmune responses, and type I, II, III, and IV hypersensitivities.

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1 The Emerging Age of Therapeutic Biologics

Since 1982 when human insulin in a recombinant form became the first recombinant protein to be approved by the US Food and Drug Administration (FDA), more than 130 proteins or peptides have been approved [1]. Following the prediction that by 2014 half of the top 10 therapeutic drugs will be monoclonal antibodies (mAbs) with sales of more than US$45 billion [2], approximately one quarter of all new drugs approved by the FDA in that year were biologics [3]. Etanercept (Enbril®), the most commercially successful fusion protein, generated worldwide sales of US$7.3 billion in 2010 and US$8.37 billion in 2012, making it the second best-selling drug after adalimumab (Humira®), one of the biggest selling drugs of all time [4]. Forecasts for 2013–2017 estimate revenue of US$58 billion for adalimumab and US$45 billion for etanercept [5], demonstrating the sales impact protein biologics have had, and are likely to continue to have, in the pharmaceutical industry since the early 2000s. By mid-2012, eight chimeric fusion proteins were on the market with FDA approval [6], namely, etanercept, alefacept, abatacept, romiplostim, rilonacept, aflibercept, belatacept, and denileukin-diftitox [6]. Apart from these fusion proteins, many others are at different stages of clinical development with some already in phase III clinical trials [7] and as basic research on, and clinical application of, cytokines with potential as fusion partners continues [8], the introduction and increasing use of new chimeric fusion proteins appears certain.

2 Chimeric Fusion Proteins

2.1 Composition

Chimeric fusion proteins used as biologic therapeutic agents [9] consist of a carefully selected and relatively short-lived effector domain, generally a peptide (a peptide is considered here to be a chain of <50 amino acids), coupled to a 'carrier', usually a protein or peptide, that also contributes to the functional properties of the resultant fusion protein. Fusion proteins are produced by genetic engineering, linking genes for the separate proteins involved to give a new polypeptide formed from the incorporated separate domains together with their functional properties [10]. The linked effector peptide may have widely varying properties, for example, contributing to recognition, binding, and toxicity, while its fused partner may aid stability and targeting of the chimeric polypeptide [11]. Effector peptides employed until now have been limited to ligand-binding portions of receptors of a few cytokines and growth factors, extracellular domains of some lymphocyte antigens, and a fragment of a protein toxin. The fusion proteins that have so far received FDA approval work as agonists of receptor function, e.g., romiplostim and alefacept; as antagonists blocking receptor function, e.g., etanercept, belatacept, abatacept, rilonacept, and aflibercept; or by a direct, targeted cytotoxic killing effect seen with denileukin-diftitox. For the future, a large number of potential candidate peptides targeting a wide variety of metabolic, cardiovascular, central and peripheral nervous system, endocrine, malignant, immunological, hematologic, allergic, and other disorders are already in clinical trials or under early investigation [4, 7, 11].

2.2 Fusion Proteins as Glycoproteins

About 70 % of purified recombinant preparations are glycoproteins and while cells such as Chinese hamster ovary cells can be engineered to express some human glycosyltransferases, variations from human glycan patterns can be obtained from a range of other organisms including bacteria, fungi, plants, insects, and mammals. For example, although *N*-glycolylneuraminic acid cannot be synthesized in humans, Chinese hamster ovary cells can add this sialic acid during glycoprotein expression [10]. For post-translational modifications, attention to the cell expression system may allow recombinant proteins to be glycosylated in a predetermined and controlled manner [12]. Glycosylation can have a major influence on the effectiveness of biologics therapy via its effects on actions, protein solubility, stability, serum half-life, immunogenicity, and selectivity of receptor binding. For example, the terminal sugars of glycans in the CH2 domain of human Fc fragments help to determine antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity [10]. Shielding by glycan structures can protect peptide sequences from proteolysis and the hydrophilic nature of the oligosaccharide chains aids solubility, helps to inhibit aggregation, and protects proteins against physical denaturation. The importance of post-translational modifications of N- and O-linked glycosylations can be illustrated by the unexpectedly rapid clearance of a Fc-fusion protein, owing to incompletely formed glycan structures with terminal *N*-acetylglucosamine or galactose on the Fc fusion partner [13].

Because of their complex nature and the limited clinical experience with biologics such as chimeric fusion proteins and biosimilars before approval, a high level of characterization is demanded for their continued development. This characterization needs to cover protein, peptide mapping, and glycan analyses and for that, state-of-the-art analytical methods for the characterization of glycoforms

need to be used. Liquid chromatography-mass spectrometry and capillary electrophoresis-mass spectrometry, as well as classical electrophoretic and chromatographic methods, are playing an increasingly important role in this respect [14–16].

2.3 Desired Properties of Fc-Fusion Proteins

As with mAbs, three main requirements in the preparation of an effective chimeric fusion protein are to endow the macromolecule with: (1) stability, that is, produce a polypeptide with a suitably extended half-life; (2) effective targeting and subsequent specific binding; and (3) cytotoxicity or at least the capacity to inhibit the deleterious processes underlying the treated condition. In efforts to achieve these properties, the crystallisable Fc region of the human IgG1 antibody has been the most commonly employed fusion partner. The Fc portion consists of the CH2 and CH3 domains of the immunoglobulin heavy (H) chain, the hinge region, and the two disulfide bridges connecting the H chains. In most chimeric fusion proteins, the C-terminus of the effector molecule, often a peptide, is fused to the N-terminus of the hinge region (Fig. 1). The effector molecule can be fused to one or both of the Fc H-chains creating a monomeric or dimeric fusion protein, respectively. Peptides have a short half-life owing to proteolytic degradation and are usually rapidly cleared via the kidneys within minutes. Conjugation to polyethylene glycol, or pegylation, can extend the half-life by increasing the hydrodynamic radius and decreasing filtration in the kidneys but safety concerns surround pegylation mainly because of a lack of biodegradability. Receptor-mediated recycling via interaction with the salvage neonatal FcRn receptor [17–19] protects Fc-containing molecules from lysosomal degradation. At low pH (<6.5) in the endosome, FcRn salvages the Fc fragment by binding, recycling, and then releasing the protein in the blood at neutral pH thus extending the Fc half-life by avoiding breakdown in the lysosomes [2, 7, 11, 18, 20]. Fc-fusion proteins also interact with Fc receptors on immune cells and have become the most frequently employed and successful structures in the preparation of chimeric fusion protein drugs with seven of the eight proteins that have been registered being Fc-fusion proteins. Half-lives of the eight proteins are listed in Table 1 where it can be seen that denileukin-diftitox, the one fusion protein lacking an attached Fc piece, has easily the shortest half-life of only about 70–80 min compared with a number of days for the Fc-fusion proteins. Note that the half-life of fusion proteins is typically significantly shorter than the half-lives of the mAbs. Alternatives to Fc fusion are emerging. Human serum albumin fusions also interact with the FcRn and, as with Fc-fusion proteins, FcRn recycling occurs by binding at acid pH and releasing at neutral pH. Transferrin is another possible fusion partner being studied [21, 22].

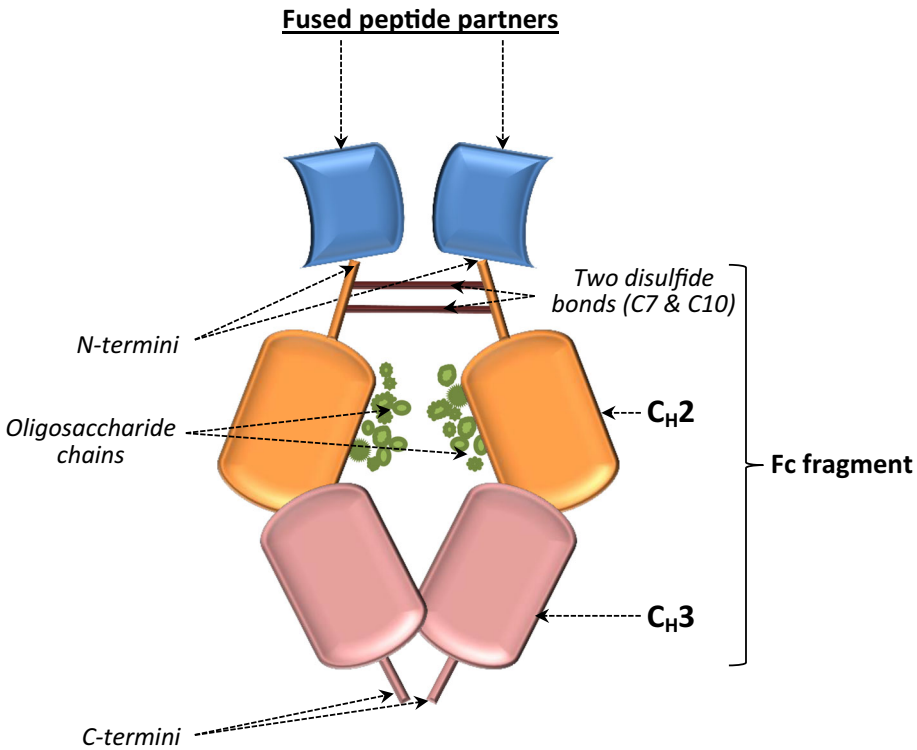

Fig. 1 Diagrammatic representation of the general structure of a chimeric human IgG Fc fusion protein linked to its effector peptide partner via the Fc N-terminus. The effector molecule(s) may be linked to one or both of the Fc chains creating a monomeric or dimeric fusion protein, respectively. In etanercept, for example, the fused peptide partner is the extracellular ligand-binding protein of the 75-kDa human tumor necrosis factor receptor

Table 1 Half-life of fusion proteins used for therapy

Fusion protein	Trade name	MW (kDa)	Half-life range in days/(h)	Average half-life in days/(h)
Etanercept[a]	Enbrel®	150	3–6	5
Belatacept	Nulojix®	90	8–10	9.2
Abatacept	Orencia®	92	8–25	16.7
Rilonacept	Arcalyst®	251	9	9
Aflibercept	Zaltrap® Eylea®	115	5–6	5.4
Romiplostim	Nplate®	60	1–34	17
Alefacept	Amevive®	92	11	11
Denileukin diftitox[b]	Ontak®	58	1.2–1.3 h	1.2 h

Data from Schmidt [20]. Fusion proteins for half-life extension

mAbs monoclonal antibodies, *TNF* tumor necrosis factor

[a] The half-life of mAbs targeting TNF (infliximab, adalimumab, golimumab, and certolizumab pegol) are approximately two to three times longer than the half-life of etanercept

[b] Not an Fc fusion protein and shows a significantly shorter half-life

2.4 IgG Subclasses of Fc Fusion Proteins: Increasing and Decreasing Effector Function

Like almost all of the mAbs approved for therapy, the Fc region used in each of the seven Fc-fusion proteins belongs to the human IgG1 subclass. The subclass used is an important consideration, especially in cancer treatment, because the biological properties of the four different IgGs differ. Human IgG1 and IgG3 bind to all FcγRs, IgG2 binds to FcγRIIA and FcγRIIIA, and IgG4 binds to FcγRI, FcγRIIA, FcγRIIB, FcγRIIC, and FcγRIIIA [23]. IgG1 (especially in non-fucosylated form) is the subclass of choice for antibody-dependent cell-mediated cytotoxicity [24] (used in, for example, rituximab, alemtuzumab, and trastuzumab), whereas human IgG2 or IgG4, which do not aid cytotoxicity, may be used when killing is not wanted [24]. Complement-dependent cytotoxicity, involving the binding of complement component C1q, activation of the complement cascade and ultimately cell death, is another mechanism by which mAbs, such as ibritumomab, kill tumor cells. IgG3 followed by IgG1 are the most effective activators of the complement cascade while IgG2 and IgG4 are relatively poor activators [25]. Sometimes fixation and stimulation of complement needs to be avoided or is unnecessary, for example, in reactions involving cytokines, and IgG2 and IgG4, which show lower affinities for Fc and complement receptors, have found use in mAbs such as tositumomab (murine IgG2 with ^{131}I for killing), denosumab (IgG2), panitumumab (IgG2), natalizumab (IgG4), and eculizumab (IgG2/4; targets complement protein C5). To date, the Fc domains of these two IgG subclasses have not been used in fusion proteins but investigations indicate that they may sometimes impart superior or optimal performance [11, 24]. The more effective activation of complement and FcγR-mediated functions of IgG3 appears to make it the subclass of choice for immunotherapy, but the antibody shows a significantly decreased half-life (~1 week compared with 3 weeks for the other subclasses) [26] and it is therefore generally not considered as a suitable Fc-fusion partner. Even so, studies with a human IgG3 containing a His at position 435 instead of the usual Arg, show that the half-life of the His435-IgG3 allotype is comparable to IgG1 [27].

3 Safety of Approved Chimeric Fusion Proteins

As outlined above, seven of the eight chimeric fusion proteins registered and approved for therapy at one time or another are Fc-fusion proteins. Table 2 summarizes the properties, mechanisms of action, approved indications, and adverse effects of the eight fusion proteins.

3.1 Etanercept

Etanercept, a recombinant, engineered, fully human, dimeric Fc-fusion protein linked to the ligand-binding portion of the human TNF receptor (Fig. 1) was the first chimeric fusion protein to gain regulatory approval when in 1998 it was approved by the FDA for the treatment of rheumatoid and other forms of arthritis. Like the mAbs infliximab, certolizumab, adalimumab, and golimumab, etanercept binds tumor necrosis factor (TNF) (Table 2) [28, 29], thereby inhibiting the interaction of this cytokine with cell surface TNF receptors and ultimately reducing the ensuing inflammatory response. As with infliximab, etanercept is sometimes administered to patients with rheumatoid arthritis when other treatments have failed and,

Table 2 Chimeric fusion proteins approved for human therapy[a]: properties, approved indications[a], mechanisms, and side effects

Generic and trade names	Properties	Approved indications[a]	Mechanism(s) of action relevant to indications	Side effects, serious and common	References
Etanercept (Enbrel®)	A dimeric fusion protein, MW 150 kDa, of the extracellular ligand-binding portion of 75 kDa human TNFR with Fc portion of human IgG1[b]	Rheumatoid arthritis; polyarticular juvenile idiopathic arthritis; psoriatic arthritis; plaque psoriasis; ankylosing spondylitis	Acts as a soluble decoy receptor for the inflammatory cytokine TNF blocking its interaction with its natural receptor	Boxed warnings: Serious infections; malignancies. Other effects: fever; injection-site reactions; cutaneous vasculitis; hypersensitivity (including anaphylaxis, angioedema, urticaria); pruritus; demyelinating disease; cytopenia; lupus syndrome	[28–135]
Belatacept (Nulojix®)	Fusion protein of Fc fragment of human IgG1 and extracellular domain of CTLA-4	Prophylaxis of organ rejection in adult patients receiving a kidney transplant[c]	Blocks CD28-mediated T-cell activation and production of cytokines[d] by binding CD80/CD86 on antigen-presenting cells	Boxed warnings: ↑ risk of PTLD[e]; ↑ susceptibility to infections and malignancies. Other effects: anemia; diarrhea; peripheral edema; hypertension; urinary tract infections; cough; hypo- and hyperkalemia; graft dysfunction	[136–146]
Abatacept (Orencia®)	Differs from belatacept by only two amino acids	Adult rheumatoid arthritis; juvenile idiopathic arthritis	As for belatacept but lower affinity for CD80/86, slower dissociation rate, and less potent and prolonged action	Infections; malignancies; immunogenicity; hypersensitivity; reactions in patients with COPD; injection-site reactions; upper respiratory tract infection; headache; nausea	[136–138] [147–158]
Rilonacept (Arcalyst®)	A dimeric fusion protein of the extracellular ligand-binding domains of IL-1RI and IL-1RAcP linked in line to human IgG1 Fc	Cryopyrin-associated periodic syndromes (CAPS)[f]	Acts as IL-1 trap blocking IL-1β signaling, thereby preventing its binding to its cell receptors and reducing inflammation	Injection-site reactions; upper respiratory tract infections; immunogenicity	[159–166]
Aflibercept (Zaltrap®[g]; Eylea®)	Fusion protein of Fc portion of IgG1 and ligand-binding domains of VEGFR1 and VEGFR2	Zaltrap: Metastatic colorectal cancer in combination with FOLFIRI. Eylea: wet macular degeneration	Acts as a soluble decoy VEGFR1 and VEGFR2 trap binding multiple isoforms of VEGF-A and placental growth factor thereby inhibiting angiogenesis	Zaltrap boxed warnings: Hemorrhage; compromized wound healing; GI perforation. Other: cytopenias; proteinuria; hypertension; ↑ serum creatinine; acral erythema; stomatitis. Eylea: eye pain; cataract; conjunctival hemorrhage; vitreous detachment; ↑ intraocular pressure	[167–176]
Romiplostim (Nplate®)	Dimeric fusion peptibody[h] MW ~60 kDa; four copies of thrombopoietin-mimetic peptide fused to the C-terminus of aglycosylated human IgG1 Fc	Thrombocytopenia in patients with chronic immune thrombocytopenia (ITP)[i]	A thrombopoietin receptor agonist. Stimulates JAK2 and STAT5 pathways → megakaryocytes and ultimately platelets	Arthralgia; dizziness; insomnia; abdominal and shoulder pain; myalgia; pain in extremity; dyspepsia; paresthesia; headache	[177–186]

Table 2 continued

Generic and trade names	Properties	Approved indications[a]	Mechanism(s) of action relevant to indications	Side effects, serious and common	References
Alefacept (Amevive®)[j]	A dimeric fusion protein MW 91.4 kDa consisting of the first extracellular domain of LFA-3 fused to human IgG1 Fc	Moderate-severe chronic plaque psoriasis in candidates for systemic therapy or phototherapy	Binds CD2 on T cells blocking interaction of CD2 with LFA on APCs thereby inhibiting T-cell activation	Warnings and precautions: Lymphopenia; malignancies; infections; hypersensitivity; hepatic injury; immunosuppression. Other effects: headache; chills; pharyngitis; urti; dizziness; cough; nausea; infections; pruritus; injection-site reactions	[187–194]
Denileukin-diftitox (Ontak®)[k]	Recombinant cytotoxic protein consisting of modified diphtheria toxin fragments (Met1-Thr387)-His and IL-2 (Ala1-Thr133) to give recombinant fusion protein[l] that binds high-affinity IL-2 receptor on leukemia cell surface	Recurrent cutaneous T-cell lymphoma with malignant cells expressing CD25	Binds to Tac subunit of IL-2 receptor on activated lymphocytes. Toxin released intracellularly causes ADP-ribosyltransferase-mediated inhibition of protein synthesis and cell death	Boxed warnings: Infusion reactions; vision disturbances, capillary leak syndrome. Other effects: pyrexia; peripheral edema; diarrhea; dyspnea; cough; nausea, rigors; pruritus; TEN	[195–201]

COPD chronic obstructive pulmonary disease; effects include exacerbation, cough, ronchi, dyspnea, *CTLA-4* cytotoxic T-lymphocyte-associated antigen 4, *FOLFIRI* folinic acid (leucovorin), 5-fluorouracil, ironotecan, *IFN* interferon, *IL* interleukin, *IL-1RacP* interleukin 1 receptor accessory protein, *JAK2* Janus kinase 2, a non-receptor tyrosine kinase, *LFA-3 (CD58)* human lymphocyte function-associated antigen 3, *PTLD* post-transplant lymphoproliferative disorders, especially of the CNS, *STAT5* signal transducer and activator of transcription 5, two related proteins STAT5A and STAT5B, *TEN* toxic epidermal necrolysis, *TNF* tumor necrosis factor, *TNFR* tumor necrosis factor receptor, *VEGFR1*, *VEGFR2*, vascular endothelial growth factor receptors 1, 2

[a] Approved by the US FDA or European Medicines Agency or both

[b] The Fc portion of IgG contains the CH2 and CH3 domains and the hinge region but not the CH1 domain

[c] To be used in combination with basiliximab induction, mycophenolate mofetil, and corticosteroids and only in patients who are EBV seropositive

[d] Blocks production of IL-2, IL-4, IFNγ, TNFα

[e] Recipients without EBV immunity are more at risk; therefore use in EBV-positive patients only

[f] Including familial cold autoinflammatory syndrome (FCAS) and Muckle–Wells syndrome (MWS) in adults and children. Note that a supplemental biologics license application for Arcalyst® for the treatment of gout flares was recommended for rejection by the FDA's Arthritis Advisory Committee in May 2012. The FDA has requested additional clinical data and details of proposed dosage

[g] Ziv-aflibercept in the USA

[h] IUPAC name: *L*-methionyl[human immunoglobulin heavy chain constant gamma-1(227 C-terminal residues)-peptide (Fc fragment)] fusion protein with 41 amino acids peptide, (7–7′; 10–10′)-bisdisulfide dimer. Lack of glycosylation negates Fc functionality

[i] Not for thrombocytopenia due to myelodysplastic syndrome or causes other than ITP

[j] In the FDA Center for Drug Evaluation and Research's (CDER) Discontinued Therapeutic Biologic Products list. Discontinued date, September 28, 2012

[k] Listed in the CDER discontinued therapeutic biologic products list January 30, 2014

[l] CD25 is the α-chain of the IL-2 receptor

as well as its indication for this disease, the protein is useful for the treatment of other autoimmune diseases including ankylosing spondylitis, plaque psoriasis and, according to some, Crohn's disease.

Early studies in particular reported that the most common adverse effects of etanercept are relatively mild, for example, fever, headache, injection-site reactions, mild allergic reactions, and pruritus [30–32] but, as so often seen after extended use over time, a wide variety of less often seen and sometimes rare and serious reactions are now known. In a retrospective safety and effectiveness assessment of etanercept given to 118 patients, 51 patients (43.2 %) experienced adverse events with one quarter being infections, mainly those of the upper respiratory tract [33]. A long-term examination of responses of 182 pediatric patients (aged 4–17 years) with plaque psoriasis revealed at least one adverse event in 80 % of the participants with upper respiratory tract infections showing the highest incidence (25 %), followed by nasopharyngitis, streptococcal pharyngitis (13 %), and sinusitis (11 %) [34]. No opportunistic infections were recorded. Continuous treatment with etanercept over 2 years of 110 patients with psoriatic arthritis recruited from 22 Canadian clinical practices, resulted in 20 serious adverse events in 14 patients. These events included abdominal abscess, appendicitis, malignant lung neoplasm, pneumonia, streptococcal infection, angina, cardiac arrest, and cerebral hemorrhage [35].

The list of etanercept adverse events issued in order of frequency by the FDA is infection, dermatologic, neurologic, musculoskeletal, pulmonary, cardiac, and vascular effects but before discussing each of these categories, it is important to point out a list of exclusion criteria formulated after careful consideration of the etanercept adverse-event profile. One such list presented on behalf of the British Society for Rheumatology Standards, Guidelines and Audit Working Group (SGAWG), refers to six different exclusion criteria [36]: (1) Women who are pregnant or breast feeding; (2) The presence of active infection; (3) Septic arthritis of a joint in the last year; (4) Sepsis of a prosthetic joint; (5) Grade 3 or 4 congestive cardiac failure; (6) A clear history of demyelinating disease.

3.1.1 Infection Risk

As referred to above, some retrospective assessments and trial results clearly show that a high incidence of infection often accompanies etanercept therapy [33, 34]. In fact, the infection risk in placebo-controlled trials has been estimated at ~35% [37] and serious infections is one of two black-box FDA warnings [38] for the drug (Table 2). Specifically, the warnings refer to: (1) Increased risk of serious infections or death due to tuberculosis, bacterial sepsis, and other opportunistic pathogens; (2) The need to discontinue the drug if infection or sepsis develops; (3) The need to perform tests for latent tuberculosis; (4) Monitor all patients for active tuberculosis during treatment. Note that the TNF-targeted mAb infliximab carries with it a three to four times higher risk than etanercept [39]. With reference to granulomatous infections associated with etanercept or infliximab where tuberculosis, histoplasmosis, candidiasis, and listeriosis make up more than 80 % of the reported cases, analysis of the reports received by the FDA's Adverse Event Reporting system revealed that of 639 episodes of granulomatous infections, 556 were associated with infliximab and 83 with etanercept [40]. In contrast to infliximab, etanercept may generally be associated with less severe infections but severe cases of viral pneumonia, pneumococcal sepsis, and osteoarticular tuberculosis have occurred [41, 42] and fatalities reported [43, 44]. The range of organisms implicated in infections associated with etanercept therapy is wide and includes bacteria (tuberculosis, streptococcus, listeria, actinobacillis) [45–50], viruses (varicella) [51, 52], fungi (aspergillus) [53], protozoa (toxoplasma) [54], and cestodes (echinococcus) [55].

3.1.2 Cutaneous Events

The most common skin manifestation seen with etanercept is an injection-site reaction (Fig. 2), which may be seen in ~20–50 % of patients [56, 57]. These are usually mild to moderate and involve erythema, edema, pain, and pruritus. Reactions occur within the first 2 months of treatment and may reoccur 1–2 days after the final injection. Interestingly, from an immunologic viewpoint, some patients develop recall reactions while continuing etanercept and skin biopsy and immunohistologic examinations revealed an inflammatory infiltrate made up mainly of lymphoid cells with some eosinophils and without evidence of leukocytoclastic vasculitis. The lymphoid cells were predominantly activated, mature, cytotoxic HLA-DR/CD3+/CD4−/CD8+ T lymphocytes. Biopsy of a recall reaction showed epidermal keratinocytes with strong expression of HLA-DR. The results were seen as consistent with a T-cell-mediated delayed hypersensitivity reaction that waned over time because of tolerance [57]. Cases of cutaneous vasculitis either as a sole manifestation of etanercept therapy [58, 59] or concomitant with other conditions such as severe glomerulonephritis [60] or accelerated nodulosis [61] have been reported and necrotizing vasculitis with eosinophils shown by biopsy and described as an autoimmune skin rash, has been described [62]. Other cases of nodules developing during etanercept therapy have also been published [63]. There are rare reports of discoid lupus erythematosus [62], clinically subacute cutaneous lupus erythematosus [64], and anti-neutrophil cytoplasmic

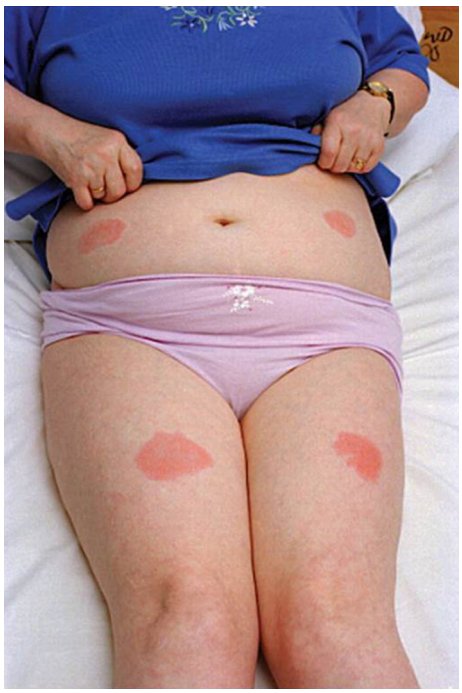

Fig. 2 Injection-site reactions at multiple sites in a rheumatoid arthritis patient treated with etanercept. Injection-site reactions are the most common skin manifestation seen with etanercept and occur in ~20–50 % of patients given the drug. (Reproduced with permission from Rajakulendran and Deighton [133])

antibody-associated vasculitis induced by anti-TNF therapy including etanercept [65].

In a comprehensive survey of 126 study reports, Lecluse et al. [66] identified 72 separate adverse skin events provoked by etanercept, making approximately 65 specific diagnoses in case studies involving 153 patients. Various rashes of unknown pathology and urticaria [67, 68] were the most commonly occurring reactions. Overall, etanercept was found to be associated with a wide variety of adverse dermatologic events, most mild but some serious and a few life threatening. Recorded cases include new onset and exacerbations of psoriasis [69–71] and atopic dermatitis [72, 73], induction of psoriasis [70, 74], lichenoid reaction [75], erythema multiforme [76], angiokeratomata [77], pemphigus vulgaris [78], and palmoplantar pustulosis [79]. There are two interesting, and rare, reports of acute generalized exanthematous pustulosis (AGEP) induced by etanercept. Within 2 days of the initiation of etanercept treatment (50 mg subcutaneously twice weekly) for severe plaque psoriasis, an adult man developed a widespread maculopapular rash. By day 4 of etanercept treatment plus antihistamines and corticosteroids, the rash had progressed to a generalized edema with tiny pustules (Fig. 3). Histologic examination revealed neutrophils, no infection, and mild spongiosis consistent with AGEP. The rash improved upon withdrawal of etanercept [80].

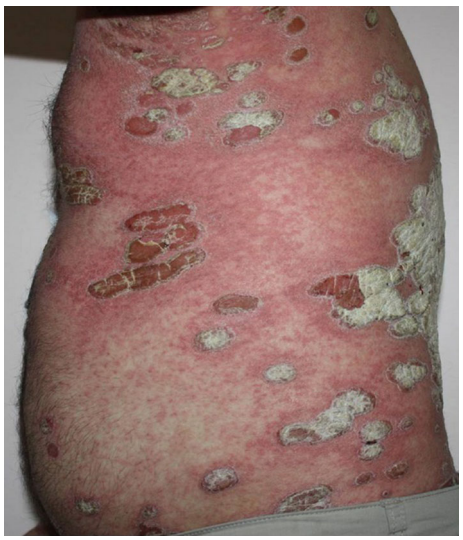

Fig. 3 Generalized erythroderma with tiny pustules in a male adult 4 days after initiation of treatment of plaque psoriasis with two injections of etanercept. Clinical features and results of histological examination were consistent with acute generalized exanthematous pustulosis. (Reproduced from Kavala et al. [80], an Open Access article distributed under the terms of the Creative Commons Attribution License.)

Following interruption and reintroduction of etanercept therapy, a 51-year-old female patient developed multiple erythematous and edematous facial lesions with small non-follicular sterile pustules accompanied by fever above 38 °C (Fig. 4a). After a few days, diffuse eythema and swelling with post-pustular desquamation spread to the limbs (Fig. 4b). Lesion morphology, the course of the disease, and histologic findings led to a clear diagnosis of AGEP [81].

3.1.3 Neurologic Events

Included in exclusion criteria for etanercept, for example by the British Society for Rheumatology SGAWG (see above), is the reminder of the risk of demyelinating disorders [36]. In an early double-blind, placebo-controlled, phase II study conducted on 168 patients, most with relapsing remitting multiple sclerosis, lenercept, now discontinued, a recombinant fusion protein similar to etanercept, combining two extracellular domains of the human p55-kDa TNF receptor with one IgG1 heavy chain, was investigated for a possible effect on induction on new lesions detected by magnetic resonance imaging scans and clinical assessments. Results showed an increase in the frequency of attacks and the severity of attacks appeared to worsen [82]. Demyelination occurring during anti-TNF therapy for inflammatory arthritides and reported to the FDA, includes 17 cases associated with etanercept. Symptoms of one case included paresthesia, optic neuritis,

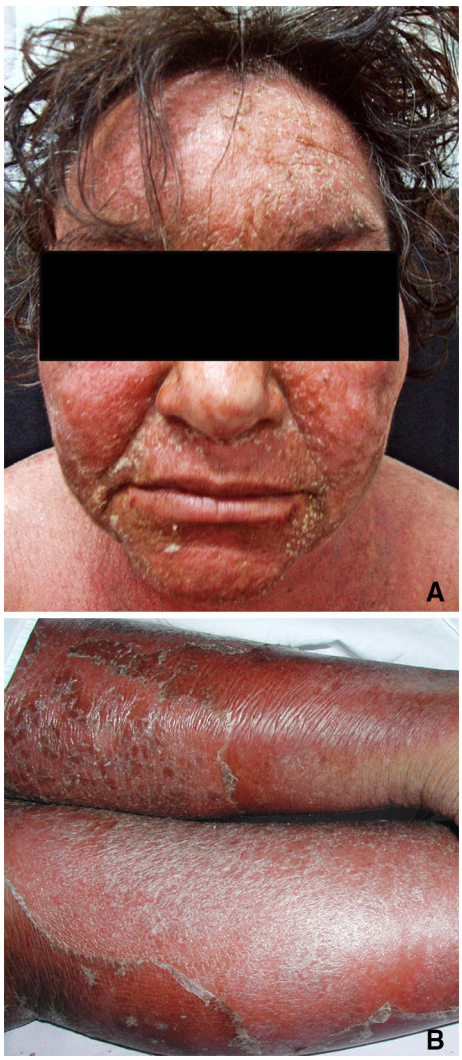

Fig. 4 **a** Edematous erythema with perioral non-infected pustules on the face of a female patient with refractory erythrodermic psoriasis and psoriatic arthritis following reintroduction of etanercept therapy. **b** Progression after a few days of diffuse erythema and swelling to the limbs showing characteristic postpustular desquamation. (Fig. 4**a**, **b** both reproduced from Vasconcelos et al. [81], an Open Access article distributed under the terms of the Creative Commons Attribution License.)

and confusion [83]. Transverse myelitis accompanied by anti-nuclear and anti-cardiolipin antibodies occurred abruptly in a 45-year-old woman 9 days after the commencement of etanercept [84] and there are reports of a number of demyelinating disorders during etanercept therapy including so-called demyelinating syndrome [85]; relapsing remitting multiple sclerosis [86]; demyelination of the spinal cord and cerebral cortex [87]; progressive multifocal leukoencephalopathy [88]; reversible posterior leukoencephalopathy syndrome [89]; demyelinating disease that resolved after withdrawal of the drug [90]; and central nervous system demyelination producing ophthalmic manifestations [91].

3.1.4 Tumorigenicity

The possibility of the development of lymphoma and other malignancies in children and adolescents treated with TNF blockers is the subject of a second FDA black-box warning [38]. A number of studies suggest an increased lymphoma risk for patients with rheumatoid arthritis, particularly those with severe disease, and the risk appears to be slightly increased in those patients treated with TNF antagonists [92]. If this is true, the cause remains poorly understood. Analysis of data on 8614 patients, 29 with lymphoma, from the US National Data Bank for Rheumatic Diseases, revealed a standardized incidence ratio (SIR) for lymphoma of 3.8 for etanercept [93]. A second cohort study of data registered in the South Swedish Arthritis Treatment Group compared 757 patients treated with etanercept with 800 patients on conventional anti-rheumatic therapy. In the former group, 16 tumors, including five lymphomas, occurred in 1603 person-years at risk (SIR 1.1, relative risk for lymphoma 11.5); in the comparison group, 69 tumors (two lymphomas) occurred in 3948 person-years (SIR 1.4, relative risk 1.3). Total tumor relative risk excluding lymphoma was 0.79 and 1.39, respectively. These results led to the overall conclusion that TNF blockers do not increase overall tumor risk in patients with rheumatoid arthritis, but may be associated with an increased risk of lymphomas [94]. As this debate continues, a number of methodologic questions about the design, execution, and analysis of the different studies have been raised. These questions include whether or not the risk following anti-TNF treatment is explained by the risk of the disease, rheumatoid arthritis itself, rather than the therapy; do all of the patients carry the same risk, e.g., those with more severe disease?; the influence of previous treatments; and is the risk related to cumulative dose and length of therapy? [95]. As well as lymphoma, there are a few reports of other malignancies associated with etanercept therapy. In the so-called Wegener's Granulomatosis Etanercept Trial Research Group study involving 180 patients with the active disease, six solid malignancies occurred. Although all patients treated with etanercept who developed tumors were also treated with cyclophosphamide, it was concluded that the combination of TNF inhibition and cyclophosphamide may increase the risk of cancer over and above the risk from cyclophosphamide alone [96]. There are a few reports of patients developing squamous cell carcinomas after treatment with etanercept including two separate cases of carcinoma of the penis [97, 98].

3.1.5 Hematologic Events

The incidence of etanercept-induced hematologic disorders is not clear. A post-marketing survey of 820 patients found an incidence of 3.4 per 1000 patient-years for patients

treated with etanercept but this figure is certain to be influenced by the treatment of many of the subjects with other anti-rheumatic drugs, particularly methotrexate [99]. After a reported 10 cases that ended in fatal sepsis, regulatory agencies issued post-marketing warnings of the possibility of pancytopenia or aplastic anemia [30]. Subsequence experience, however, has shown hematologic cases occur only rarely, for example, a case of reversible aplastic anemia after 16 weeks of etanercept therapy [100] and myelopoiesis diagnosed as exacerbation of macrophage activation syndrome in a young woman with adult-onset Still's disease. After multiple transfusions, intravenous immunoglobulin and granulocyte-macrophage colony-stimulating factor, she was successfully treated with methylprednisolone and cyclosporin [101]. Of 267 patients with rheumatoid arthritis, ankylosing spondylitis, or psoriatic arthritis receiving etanercept therapy, 49 (18.4 %) developed at least one episode of neutropenia, although only about 1 % of patients developed severe infections secondary to neutropenia [102]. Other reports of cytopenias following the fusion protein include cases of neutropenia [103, 104], leukopenia and thrombocytopenia [105], and leukopenia alone [106]. In 2010, the British Society of Rheumatology updated their consensus guidelines recommending regular complete blood cell counts for patients undergoing TNF inhibitor therapy [107].

3.1.6 Respiratory Events

Possible nodulosis after etanercept is well known (see Sect. 3.1.2 above and [61, 63]), but pulmonary nodulosis is an unusual manifestation of rheumatoid disease [108]. Pulmonary nodules have been diagnosed after etanercept treatment but it is not always necessary to discontinue the drug [109]. The condition demonstrates the need for careful monitoring of the drug treatment and for testing to achieve a differential diagnosis of tuberculosis. Pulmonary granulomas after etanercept may also be difficult to distinguish from other lung complications including tuberculosis. Infection as a cause was not ruled out in two cases examined by lung biopsy—non-caseating granulomas containing birefringent particulates in one [110] and caseating necrosis in the other [111]. Two patients, both previously given methotrexate and with pre-existing lung disease, developed acute respiratory symptoms within 3–6 weeks of beginning etanercept therapy. Rapid deterioration into accelerated interstitial lung disease ensued and one patient died despite aggressive treatment. The authors concluded that caution is needed with patients with rheumatoid arthritis taking methotrexate and with pre-existing lung disease when etanercept is added [112]. Three other cases of exacerbation of pre-existing interstitial lung disease after administration of etanercept [113, 114] and a case of organizing pneumonia in a patient with rheumatoid arthritis treated with etanercept have been described [115].

3.1.7 Immunologic Events

Any discussion of immunologic events associated with etanercept therapy, and in fact any therapy with protein biologics [116], needs to recognize that many events considered under a range of different headings, for example, cutaneous, hematologic, pulmonary, renal, endocrine, and probably others, are either already known to have an immune basis or component or such an association is suspected. Thus, a number of dermatologic events provoked by the fusion protein and diagnosed as psoriasis, atopic dermatitis, erythema multiforme, and acute generalized exanthematous pustulosis are almost certainly type IV hypersensitivity responses. Likewise, the occasional recall skin responses and identification of CD8+ cytotoxic T cells seen in injection-site reactions [57] indicate a delayed hypersensitivity mechanism while some early responses in such reactions may be true immediate, type I hypersensitivities. At least some cases of etanercept-induced cytopenias and vasculitis may be type II and type III hypersensitivities, respectively, and some pulmonary events caused by etanercept may ultimately be shown to be type III or combined type III/type IV reactions. There are increasing indications that etanercept can provoke autoimmune reactions such as hyperthyroidism [117] and the development of anti-synthetase syndrome [118], Crohn's disease [119], and Henoch-Schönlein purpura. In one case of the latter, increased levels of IgA rheumatoid factor resulted in IgA immune complexes [120]; in another case, Henoch–Schönlein purpura occurred with acute renal failure [121]. In fact, biologic-induced autoimmune renal disorders are being increasingly recognized especially in relation to etanercept, which has been the biological agent most frequently identified with the condition [122]. A total of 20 vasculitic adverse events after etanercept, particularly hypersensitivity vasculitis and necrotizing vasculitis, were recorded by the FDA Adverse Events Reporting System. Most cases developed within 3 months [123] but evidence to establish underlying mechanisms of the reactions was generally not sought, let alone published. This subject, together with the question of whether some adverse reactions to etanercept and other fusion proteins are true type I, II, III, or IV hypersensitivity responses is taken up below in the Sect. 5.

The occurrence of anti-etanercept antibodies is well known but there appears to be no evidence to date that these antibodies reduce the clinical efficacy of the drug [116]. The antibodies occur with frequencies of 3–5.6, 0, 8, and 18 % in rheumatoid arthritis, ankylosing spondylitis, children with juvenile idiopathic arthritis, and psoriasis,

respectively [7]. It has been known for some time that patients given etanercept commonly develop anti-nuclear and/or anti-double-stranded DNA antibodies and cases of a lupus-like syndrome, cutaneous lupus erythematosus (Fig. 5), and acute discoid lupus have been reported [124, 125]. In at least one patient, the anti-nuclear and anti-DNA antibodies appeared to be associated with treatment failure. There appears to be at least a half dozen possible/probable reports of immediate hypersensitivity/anaphylaxis to etanercept [126], including two cases of anaphylaxis in children with juvenile idiopathic arthritis [127] and two episodes of angioedema [128, 129]. In two other reported cases, one patient experienced urticaria and swelling of the tongue and periorbital regions within hours of administration of etanercept; the second patient, who had Still's disease, experienced facial swelling, periorbital edema, diffuse pruritic rash, and difficulty swallowing, again within hours of the injection [126]. In a study of hypersensitivity reactions to the anti-TNF agents infliximab, adalimumab, and etanercept, Puxeddu et al. [130] found nine patients with reactions to etanercept, five with urticaria/angioedema, and four local reactions. Positive intradermal tests to the drug were seen in two of the five patients with urticaria/angioedema and three of the patients with local reactions. Two patients were found to react to etanercept and both mAbs; one proved skin test positive only to infliximab, the second positive to all three agents. Although an injection-site reaction is the most common adverse event of etanercept therapy, the mechanism(s) of the reaction(s) has not been well studied and suggestions that at least some of the reactions are type I hypersensitivities is not always supported by definitive evidence. Positive skin tests to etanercept, both prick and intradermal, were obtained in two patients treated with the drug [131] but to date there are no reports of specific IgE antibodies. A patient who developed a severe generalized pruriginous exanthema 2 h after receiving etanercept proved patch and prick test-negative to the agent but reacted positively upon intradermal testing [132]. As noted [57], some injection-site reactions, sometimes at multiple sites [133], may be delayed hypersensitivities mediated by CD8+ lymphocytes but biopsies from two patients with rheumatoid arthritis who developed recall reactions during etanercept therapy revealed a predominance of CD4+ T cells in the inflammatory infiltrate [134]. In another variant finding, biopsy specimens from a pruritic erythematous edematous reaction on the thigh of a woman treated with etanercept demonstrated papillary edema and a polymorphous infiltrate with a predominance of eosinophils and scattered flame figures. This was interpreted as a case of eosinophilic cellulitis proceeding via a T helper 2-mediated response [135].

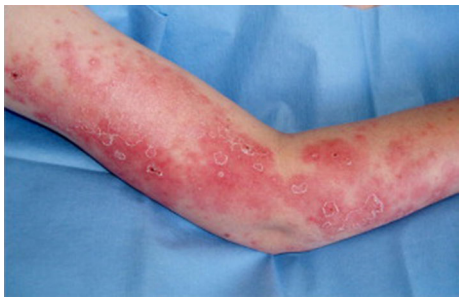

Fig. 5 Erythematous macular eruption on the arm of a patient diagnosed with cutaneous lupus erythematosus induced by etanercept. (Reproduced with permission from Abourazzak et al. [125])

3.2 Belatacept

3.2.1 Origin, Nature, Mechanism of Action, and Use

Interference with, or prevention of, T-cell co-stimulation can be an effective strategy for immunomodulation and this approach has been used in the development of two Fc-fusion proteins abatacept and belatacept (Table 2). For naïve T cells to be activated, two signals are required: signal 1 from an antigenic peptide, major histocompatibility complex expressed on antigen-presenting cells, which interacts with the T-cell receptor; and signal 2, the so-called co-stimulatory stimulus, which is antigen non-specific and activated when B7-1 (CD80) and B7-2 (CD86) on the surface of dendritic cells bind CD28 on T cells [136]. CD80/86 can also bind a homolog of CD28, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), which in fact binds with higher affinity. Given the high affinity of CTLA-4 for CD80/86, the former was used as the peptide partner to prepare abatacept by fusing the extracellular domain of the lymphocyte antigen to the N-terminal of the Fc fragment of human IgG1 (Fig. 1) to increase the half-life of the chimeric fusion protein [137]. The rationale for this approach was to block stimulation of T cells via CD28 and although abatacept proved efficacious for some T-cell-mediated autoimmune disorders such as rheumatoid arthritis, it proved less efficacious as an immunosuppressant in transplantation. This was because although abatacept showed high affinity for CD80/86, CTLA-4 is a much less potent inhibitor of CD86-dependent than CD80-dependent co-stimulation [138]. This information led to the realization that a modified CTLA-4 molecule with higher avidity for CD86 should be sought. Codon-based mutagenesis and surface plasmon resonance studies identified amino acid residues 24 and 104 as critical for binding. The most avid molecule, later named belatacept, differed from abatacept by two amino acid substitutions (L104E, A29Y). It bound four times more avidly to CD86, resulting in an overall 10-fold increase in biological activity compared with CTLA-4 [139].

Belatacept acts as an immunosuppressant reducing reliance on calcineurin inhibitors and corticosteroids and appears to show better allograft function and improved cardiovascular and metabolic risk profiles than cyclosporin [140–142]. This has led to its approval by the FDA for prophylaxis of organ rejection in adult kidney transplant patients [143].

3.2.2 Safety of Belatacept

Approximately 20 % of patients undergoing therapy with belatacept develop adverse effects, the most common of which are listed in Table 2. In the two major phase III trials termed BENEFIT [141] and BENEFIT-EXT [142], acute infusion reactions, defined as a reaction within the first hour of an infusion, occurred in a total of 24 of the 804 patients (3 %) receiving the more intense and less intense belatacept dosage regimens. All reactions were mild to moderate except for one, which was a serious prolonged hypotensive event. Data on infections from the phase III trials revealed mild to severe urinary tract infections in 263 of 949 patients (27.7 %) receiving the drug, upper respiratory infections in 8.5 %, cytomegalovirus in 10.1 % (compare 11.7 % in cyclosporin patients), and pneumonia in 2.5 % of patients [141, 142]. Immunosuppression with belatacept-based and corticosteroid-avoiding regimens in de novo kidney transplant recipients was studied in a 1-year controlled open-label study in which recipients of renal allografts were randomized to receive belatacept-mycophenolate mofetil, belatacept-sirolimus, or tacrolimus-mycophenolate mofetil. Infection of any sort was seen in 79, 77, and 67 %, and a serious infection in 21, 15, and 17 % of patients in the three different groups, respectively [140]. These findings indicate that with respect to infection rate, belatacept compares favorably to cyclosporin. However, in addition to an FDA black-box warning stating that "only physicians experienced in immunosuppressive therapy and management of kidney transplant patients should prescribe Nulojix", the warning also states that "increased susceptibility to infection…may result from immunosuppression" and the drug's use in liver transplant patients is not recommended because of an increased risk of graft loss and death [143].

Fatal progressive multifocal leukoencephalopathy occurred in one kidney and one liver transplant patient receiving the more intense belatacept regimen, leading to this condition being included in the FDA's 'Warnings and precautions' for the fusion protein (Table 2) [144]. In the two phase III belatacept trials, 11 out of 445 patients (2.5 %) in one trial [141] developed malignancy and 8 out of 359 (2.2 %) developed malignancy in the other trial [142]. Comparative incidences for cyclosporin were 0.5 and 3.3 %. The possibility of the development of malignancies is included in the current FDA black-box warning for belatacept (Table 2) [143]. Of additional concern was the diagnosis of five cases of post-transplant lymphoproliferative disorder (PTLD) (two involving the CNS) in the BENEFIT trial and five cases (five involving the central nervous system) in the BENEFIT-EXT trial. Taking into account the major trials and the follow-up period, 13 cases of PTLD occurred, while for cyclosporin only two cases (0.4 %) were recorded. Interestingly, Epstein-Barr virus (EBV or human herpesvirus 4) seronegativity is a risk for developing PTLD from belatacept therapy for kidney transplantation. EBV-seronegative transplant recipients developed PTLD with an incidence of 7.3 % compared with 0.6 % for EBV-seropositive patients [145]. The importance of a transplant recipient's EBV-serum antibody status appears to be borne out by the apparent absence of cases of PTLD in a trial excluding EBV-seronegative transplant recipients [140]. In June 2011, the FDA issued a document entitled Risk Evaluation and Mitigation Strategy (REMS) for Nulojix® in which the stated goals were to inform healthcare providers of the increased risk of post-transplant PTLD, predominately in the CNS, associated with Nulojix®; the increased risk of PML associated with the drug; and to inform patients of serious risks associated with Nulojix®. To ensure the effectiveness of the REMS, the provision of a medication guide with each Nulojix® infusion and a communication plan for healthcare workers [146] were required. PTLD and the relevance of EBV-negativity/positivity are also included in the current FDA black-box warning [143].

Development of antibodies to belatacept was assessed in 372 treated patients, many for up to 2 years. Of 29 who tested positive, 13 had antibodies to the modified CTLA-4 fusion protein. Anti-belatacept antibodies were not implicated in altered clearance of the drug [143].

3.3 Abatacept

Abatacept (Table 2), an Fc-fusion protein containing the extracellular domain of CTLA-4 (for development, description, and mechanism of action see above under belatacept), is approved as a first-line treatment of adult rheumatoid arthritis and juvenile idiopathic arthritis [147]. However, preliminary results indicate that the drug may also have a role in the treatment of other autoimmune diseases such as psoriatic arthritis and psoriasis [148].

3.3.1 Adverse Events Identified in Clinical Trials

The most frequently reported adverse reactions to abatacept recorded in a number of trials include nasopharyngitis, headache, nausea, diarrhea, upper respiratory tract infections, and arthralgia. The safety of abatacept was assessed

in multicenter, randomized, double-blind, placebo-controlled studies of patients with methotrexate- or anti-TNF-resistant rheumatoid arthritis [149–151]. Acute infusion reactions occurred more frequently in the abatacept-treated groups than the placebo groups, although there was no apparent relationship between serious reactions and the number of infusions [149, 150]. Two patients discontinued participation because of a hypersensitivity rash and one discontinued because of hypotension [151]. Infections, particularly pneumonia, nasopharyngitis, sinusitis, upper respiratory infections, and bronchitis, were seen more often in the abatacept groups [149, 151]. The incidence of benign and malignant neoplasms and hematological disorders was similar in the drug and placebo groups and no major autoimmune disorders such as lupus or multiple sclerosis occurred [151]. Immunogenicity studies found only small numbers of patients developed antibodies to abatacept—six (1.4 %) in one trial [151] and, in another, 3 of 234 (1.3 %) with one against the Fc portion and two against CTLA-4 [151]. An integrated safety analysis carried out on the abatacept clinical trials assessed results on 1955 patients treated with the fusion protein during the double-blind periods and 2688 during the cumulative double-blind and open-label periods, yielding 4764 patient-years of exposure in total. Acute infusion reactions were mostly mild to moderate, the overall frequencies of adverse events, serious adverse events and malignancies were similar in the treated and control patients and abatacept was associated with low immunogenicity with no associated safety or efficacy issues [152]. In a similar safety assessment that included open-label long-term extension of exposure (3–5 years) data, incidence rates defined as events/100 patient-years were determined for serious events (8.76), infections (44.8), serious infections (1.72), malignancies (1.19), and autoimmune events (1.31). Twenty-seven patients (2 %) experienced injection-site reactions, all except one of which were mild [153].

3.3.2 Other Reported Adverse Events

Adverse skin reactions to abatacept appear to be rare but there are a number of cases of paradoxical psoriasiform eruptions to the agent. A meta-analysis of trials in which rheumatoid arthritis patients received abatacept as monotherapy (1332 patients), or together with other anti-rheumatic drugs (1945 patients), revealed 4 (0.3 %) and 13 (0.67 %) cases of psoriasis, respectively [154–156]. A case of erythema elevatum diutinium has been reported in a juvenile idiopathic arthritis patient treated with abatacept [157].

The recent interesting observations that abatacept produced partial or complete remissions of proteinuria in five patients with focal segmental glomerulosclerosis (FSGS), that four of the patients had recurrent FSGS after transplantation, and patients had proteinuria with B7-1 immunostaining of podocytes in kidney biopsy specimens, suggests that B7-1 may be a useful marker for the treatment of some glomerulopathies [158].

3.4 Rilonacept

Rilonacept is a dimeric Fc-fusion protein in which the interleukin (IL)-1R accessory protein (IL-1RAcP) ligand-binding region is fused via its C-terminus to the N-terminus of the interleukin receptor IL-1RI extracellular domain and these linked peptides are then fused via IL-1RI to the N-terminus of each of the Fc chains of human IgG1 (Fig. 6). Rilonacept, also known as IL-1 trap (target-related affinity profiling), captures IL-1β preventing activation of IL-1 receptors and thus reducing the inflammation and other effects due to overproduction of IL-1 (Table 2) [159–161]. Rilonacept was granted orphan drug status and approved for the treatment of cryopyrin-associated periodic syndromes (CAPS), a group of rare inflammatory diseases with an incidence of about one in one million in the USA. CAPS is generally caused by mutations in the NLRP-3 [nucleotide-binding domain, leucine rich family (NRL), pyrin domain containing 3] gene. Cryopyrin regulates caspase-1, which controls the activation of IL-1β involved in activation of the immune and inflammatory responses. Mutations in NLRP-3 lead to excess release of IL-1β and the resultant inflammatory symptoms seen in CAPS.

Perhaps reflecting its orphan drug status and therefore consequent lighter use, the list of adverse events provoked by rilonacept is relatively short compared with most therapeutic biologics (Table 2). Clinical trials, as well as results of a 72-week open-label extension study in patients with CAPS, including familial cold autoinflammatory syndrome and Muckle–Wells syndrome, revealed few concerning adverse effects. Adverse events were generally mild to moderate, the most common being injection-site reactions and upper respiratory tract infections. Other reactions observed included sinusitis, cough, nausea, diarrhoea, hypoesthesia, and urinary tract infections [162, 163]. A phase III, randomized, placebo-controlled trial of rilonacept for gout flare prevention also concluded that these two events were the most frequently occurring reactions. No clear increase was seen in rilonacept-associated infections and no tuberculosis or opportunistic infections were reported. Gout exacerbation and neutropenia were responsible for two discontinuations from the trial [164]. Headache and dizziness were the most common adverse events in a randomized controlled trial of rilonacept in the treatment of acute gouty arthritis [165]. Warnings and precautions for rilonacept issued by the FDA state that IL-1 blockade may interfere with the immune response to

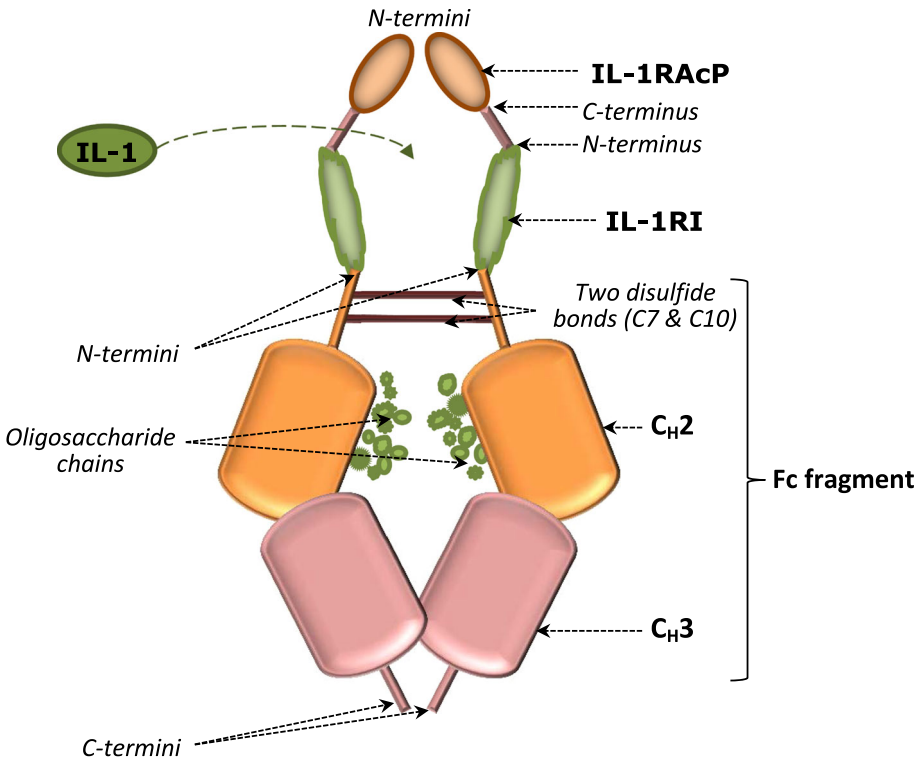

Fig. 6 Diagrammatic representation of the structure of the dimeric human IgG1 Fc fusion protein rilonacept or 'IL-1 trap', which captures IL-1β preventing activation of IL-1 receptors. *IL* interleukin

infections and that live vaccines should not be given concurrently with the drug. The impact of treatment with rilonacept on the development of malignancy is not known. Immunogenicity examinations showed that 19 of 55 (35 %) subjects who received rilonacept for at least 6 weeks developed antibodies to the fusion protein but no correlations between the presence of antibodies and clinical and safety effectiveness were found [166].

3.5 Aflibercept

Aflibercept, or VEGF trap, is a human recombinant protein made by fusing domain 2 from vascular endothelial growth factor receptor-1 (VEGFR-1) to domain 3 of VEGFR-2 and attaching this combination to the hinge region of the Fc domain of human IgG1. The VEGF trap acts as a circulating antagonist preventing receptor binding by VEGF and placental growth factor (PIGF) [167].

Aflibercept, as ziv-aflibercept or Zaltrap®, is used in combination with 5-fluoruracil, leucovorin, and ironotecan (FOLFIRI) for the treatment of oxaliplatin-resistant metastatic colorectal cancer [168] and, as Eylea®, as an ophthalmic intravitreal injection for the treatment of neovascular (wet) age-related macular degeneration and for macular edema following central retinal vein occlusion [169].

Early clinical trial results with aflibercept in patients with advanced solid tumors showed that the most common adverse events were fatigue and nausea/vomiting and toxicities associated with treatment were dysphonia, hypertension, and proteinuria. No patients developed antibodies to the fusion protein [170]. In a phase III multicenter, randomized, controlled trial designed to compare aflibercept/FOLFIRI with placebo, adverse events led to discontinuation of 26.6 and 12.1 % of patients in the aflibercept and placebo groups, respectively. Grade 3 and 4 adverse events in the patients receiving aflibercept with a more than 5 % incidence compared with placebo were, in order of highest to lowest incidence, neutropenia, hypertension, diarrhea, asthenia, stomatitis, infections, and proteinuria. Febrile neutropenia showed an incidence of 3.9 %. A 13-fold increase was seen in the hypertension rate and a 6.5-fold increase in the proteinuria rate [171, 172]. As set out in Table 2, warnings, precautions, and known adverse effects of ziv-aflibercept are quite extensive. A black-box warning covers potentially fatal hemorrhage including gastrointestinal hemorrhage, gastrointestinal perforation, and compromised wound healing. Other warnings and precautions draw attention to fistula formation, hypertension, arterial thrombotic events, proteinuria, neutropenia and its associated complications, diarrhea and dehydration and reversible posterior leukoencephalopathy syndrome (RPLS) [168]. A recent, phase II multicenter lung cancer trial of ziv-aflibercept with cisplatin and pemetrexed in 42 patients was closed prematurely because of three confirmed and two suspected cases of RPLS [173].

Although RPLS has been noted in other ziv-aflibercept clinical assessments, the rate observed in this study was higher than previously seen. The incidence of antibodies to ziv-aflibercept after its intravenous administration was found to be 3.1 %. Neutralizing antibodies were found in 17 of 48 patients but their impact, if any, on the efficacy and safety of the drug was not assessed.

Apart from the necessary warnings and precautions associated with intravitreal injections, the FDA prescriber's information for the aflibercept ophthalmic preparation Eylea® draws attention to a potential risk of arterial thrombotic events following the intravitreal use of VEGF inhibitors [169]. The most common adverse reactions occurring with a frequency of ≥5 % are listed in Table 2. Much of the data on adverse events were obtained in clinical trials designed to evaluate the efficacy and safety of aflibercept injection in the treatment of wet age-related macular degeneration and macular edema secondary to central retinal vein occlusion. In the studies on both of these conditions, immunoreactivity to the drug was 1–3 %. Again, no differences in efficacy or safety were seen in patients with or without antibodies [174–176].

3.6 Romiplostim

Romiplostim, a ~60-kDa so-called peptibody [177], is formed by the fusion of four identical copies of a thrombopoietin mimetic peptide to the C termini of aglycosylated human IgG1 Fc chains produced in *Escherichia coli*. Each H chain of the Fc-protein is attached at residue 228 by a pentaglycine bridge to a molecule of the thrombopoietin mimetic peptide linked to another molecule of the peptide by an octaglycine bridge (Fig. 7). Note that unlike the Fc-fusion proteins reviewed above, the biologically active peptide of romiplostim is not linked to the *N*-terminal end of the Fc fragment. The peptide employed was identified by screening phage libraries and optimized for its capacity to displace thrombopoietin from its receptor hTPOR and to stimulate the proliferation of cells specifically engineered to express hTPOR [178].

As a thrombopoietin receptor agonist, romiplostim is indicated for the treatment of thrombocytopenia in patients with chronic immune thrombocytopenia who have had an insufficient response to corticosteroids, immunoglobulins, or splenectomy. It is not used for any cause of thrombocytopenia other than immune thrombocytopenia [179, 180]. In the main, adverse events in clinical trials were rated mild to moderate (Table 2) [181–183] but there are regulatory agency warnings for thrombotic/thromboembolic complications, bone marrow reticulin formation [184], and bone marrow fibrosis following the occurrence of cases in clinical trials [181, 182, 185]. A warning has also been issued of the risk of progression of myelodysplastic syndromes to acute myelogenous leukemia [179]. Adverse events seen in a trial with children were mild to moderate with reports of headache, cough, vomiting, and epistaxis. Thromboembolic events were not seen [186]. No correlation between antibody activity (pre-existing antibodies or antibodies developed during treatment) and clinical effectiveness or safety has been observed.

3.7 Alefacept

Interaction of CD2 with human cell surface LFA-3 (lymphocyte function-associated antigen 3; CD58) produces signaling events involved in the regulation of T-cell responses (Table 2). In 1993, Miller et al. [187] localized the CD2 binding site to the first domain of LFA-3, used it as the effector peptide to prepare a fusion protein with the Fc fragment of IgG1 and showed that this N-linked fusion protein could inhibit cell proliferation and mixed lymphocyte reactions. Alefacept, which can be viewed as an anti-CD2 (Table 2), selectively targets effector memory T cells for both CD4+ and CD8+ but not naïve T cells and central memory T cells in psoriasis vulgaris. The increased expression of CD2 on effector T cells, their decrease induced by alefacept, and their concentration in psoriatic skin lesions suggest a role for the effector T cells in disease pathogenesis [188]. However, although circulating memory T cells decrease in all alefacept-treated psoriasis patients, a good correlation does not exist between clinical response and circulating memory T-cell reductions. The mechanism of T-cell reduction also remains to be established.

The safety and tolerability of alefacept has been detailed in a number of clinical trials where the most common adverse effects, including injection-site reactions, were mild to moderate (Table 2) and there appeared to be no correlation between decreased CD4 + T-cell counts and the incidence of infections [189–191]. An analysis of 13 trials examining the long-term safety of alefacept in 1869 psoriasis patients who had received up to nine courses of therapy [192], showed a similar spectrum of adverse events and their incidences, for example, headache (0–14.2 %), nasopharyngitis (7.7–25 %), influenza (0–8.1 %), upper respiratory tract infection (0–12.5 %), and pruritus (0–7.5 %). Less than 1 % of patients developed an infection, no opportunistic infections were seen, and infections that did occur appeared to be unrelated to CD4+ T-lymphocyte counts. Incidences of serious adverse events (0–4.8 %), serious infections (0–0.9 %), malignancies (0–4.8 %), and discontinuations due to adverse events (0–4.8 %) showed no apparent increase with repeated exposure. Four of 1869 patients (0.2 %) experienced angioedema while urticaria occurred in six patients. Lymphopenia with reductions in CD4+ and CD8+ lymphocytes, malignancies, serious infections, and

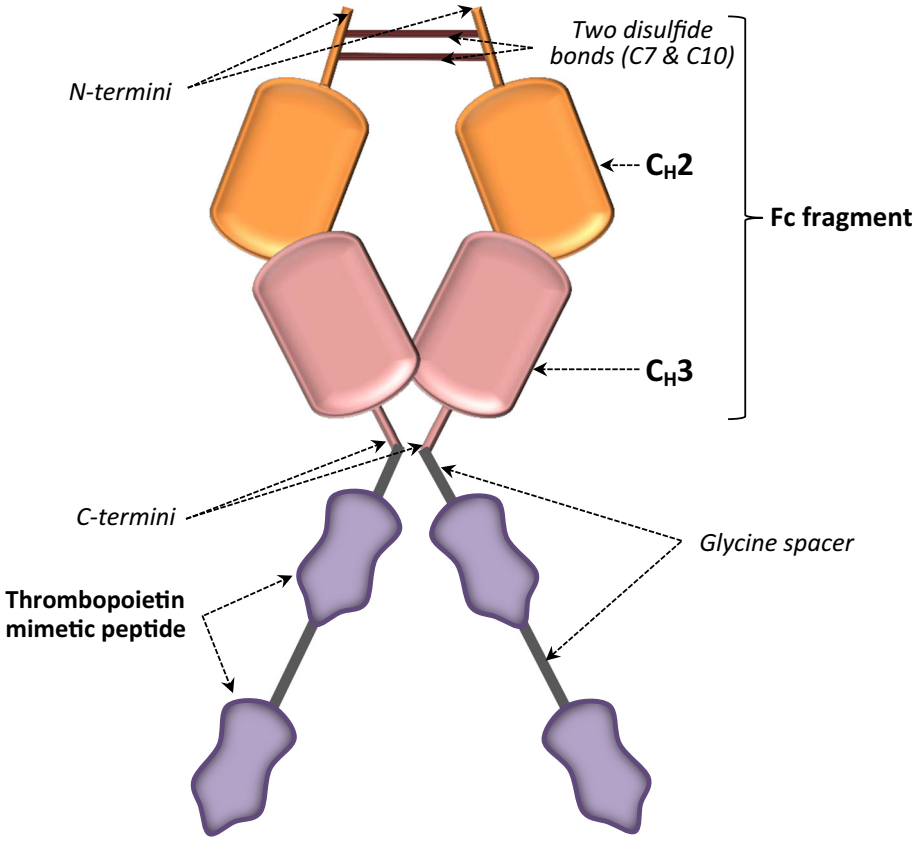

Fig. 7 Diagrammatic representation of the structure of the dimeric human IgG1 Fc fusion protein romiplostim, which acts as a thrombopoietin receptor agonist. Note that unlike the other six approved chimeric Fc fusion proteins, the effector peptide partner (two linked thrombopoietin mimetic peptide molecules attached to each Fc H-chain) of romiplostim is linked to the C-terminal, not the N-terminal, end of the Fc fragment. Note also that the oligosaccharide chains normally attached to the IgG1 C_H2 domains are absent in romiplostim

hypersensitivity reactions are each the subject of warnings and precautions by the FDA (Table 2) and each are listed as the most serious adverse reactions of alefacept [193]. Thirteen malignancies were detected in 11 alefacept-treated patients in a placebo-controlled study. The incidence of malignancy was 1.3 and 0.5 % in the placebo group. Hepatic injury caused by the drug is also listed in post-marketing reports and the potential for excessive immunosuppression is another warning issued by the FDA. In this respect, it has been suggested that alefacept should be used with caution in patients with known mycosis fungoides or an unclassified atypical lymphocytic skin infiltrate [194]. Approximately 3 % of 1357 patients developed low-titer antibodies to the fusion protein but the long-term effect, if any, of these antibodies is not known [193]. After a decision by the manufacturer to cease production of Amevive®, FDA approval for alefacept was discontinued in September 2012. The decision does not seem to have been associated with any safety or risk concerns. The drug was never approved for the European market.

3.8 Denileukin-diftitox

This genetically engineered recombinant fusion protein introduced in 1992 as DAB389IL2 was the first fusion toxin to be approved. Made up of the full-length IL-2 molecule and the catalytic domain of diphtheria toxin, that is a single polypeptide chain of 388 amino acids obtained by deleting the 147 amino acid receptor-binding domain from the 535 amino acid full-length diphtheria toxin, the resultant fusion protein retains the ADP-ribosyltransferase and membrane translocating domains of native diphtheria toxin (Table 2) [195, 196]. Once bound to the IL-2 receptor (IL-2R), the fusion toxin needs to undergo endocytosis to effect cell killing by inhibiting protein synthesis. This is achieved only by binding to cells that have intermediate- or high-affinity IL-2Rs. Human IL-2Rs may have low, intermediate, or high affinity. High-affinity IL-2R ($K_d \sim 10^{-11}$) results from the complex of three different proteins, the α chain of MW 55 kDa (CD25, p55, TAC), β chain of MW 75 kDa (CD122, p75), and the γ chain, MW 64 kDa (CD132, p64). By itself, CD25 acts as the low-affinity receptor; CD122 and CD132 together function as an IL-2R receptor of intermediate affinity ($K_d \sim 10^{-9}$) [197].

Perhaps the most extensive examination and analysis of safety data for denileukin diftitox is contained in the results of the pivotal phase III trial of the fusion protein for the treatment of cutaneous T-cell lymphoma [198]. In this study, adverse effects were first seen, and occurred in most patients, during the first treatment course. In the first 24 h, acute hypersensitivity-like reactions involving dyspnea, hypotension, chest tightness, and back/chest pain occurred

and approximately one third of patients experienced cutaneous infusion-related events including flushing and pruritus. Note that the possibility of serious and even fatal infusion reactions is included in an FDA black-box warning for denileukin-diftitox (Table 2) [199]. Temporary interruption of treatment, a decrease in the infusion rate, and/or the administration of antihistamines and corticosteroids can be used to alleviate or prevent the acute symptoms. The most frequently seen adverse events were flu-like symptoms (55 of 65, 85 %) and gastrointestinal symptoms (65 of 71, 92 %) consisting of chills, fever, headache, nausea/vomiting, diarrhoea, asthenia, myalgia, arthralgia, and anorexia. Vascular leak syndrome, also included in the FDA black-box warning, usually seen in the first 14 days of treatment, and defined as at least two of edema, hypoalbuminaemia, and/or hypotension, was reported by 25 % of patients. Infections occurred in 56 % of patients but were considered typical of advanced-stage, cutaneous T-cell lymphoma patients and unrelated to treatment. Leukopenia, neutropenia, and thrombocytopenia were reported in from one to three of the 71 patients and although lymphopenia occurred in 70 % of patients, 24 % had lymphopenia at baseline. Rashes not related to infusions manifested in 25 % of patients but overall 35 % had cutaneous reactions classified as maculopapular, petechial, vesicular-bullous, urticarial, and/or eczematous. Cutaneous reactions classified as delayed hypersensitivities and including a case of exfoliative dermatitis, were reported in 3 of 35 patients with psoriasis participating in a denileukin-diftitox dose-escalation study [200]. There has been at least one report of toxic epidermal necrolysis to denileukin-diftitox; the case proved fatal [201]. Results of two studies of the immunogenicity of denileukin-diftitox have been summarized by the FDA [199]. In the first, 66 % of 95 treated patients tested positive for antibodies at baseline probably owing to previous exposure to a diphtheria organism or vaccine. By treatment courses 1, 2, and 3, the percentage had risen to 94, 99, and 100 %, respectively, while pharmacokinetic parameters decreased significantly and clearance increased two- to eight-fold. In the second study, 39 % of 131 patients had antibodies at baseline and this increased to 66 % after one course of treatment and 97 % after three courses. Assessment of neutralizing antibodies showed that inhibited functional activity increased from 45 % at baseline to 97 % after three courses. In the pivotal phase III trial [198], the authors concluded that development of antibodies to the fusion toxin did not appear to impair the response to treatment and no clinical correlation was observed between levels of antibodies to IL-2 and any adverse event. In fact, higher levels of antibody to denileukin-diftitox were associated with lower incidences of rash and hypoalbuminemia and higher transaminase levels. Loss of visual acuity, with or without retinal pigment mottling, is a third possible adverse event listed in the FDA black-box warning. Although some patients have recovered, visual impairment persisted in most [199]. Ontak® was included in the Center for Drug Evaluation and Research discontinued biologic products list in January 2014.

4 The Immunogenicity of Therapeutic Fusion Proteins: Attempts to Help Recognize Patients at Risk

The possibility of an immune response to a therapeutic protein must always be kept in mind because such a response has the potential to adversely affect the safety as well as the efficacy of the treatment by inhibiting or blocking the protein's therapeutic action. There are a number of immunologically based or influenced adverse events that can eventuate after administration of a fusion protein such as anaphylaxis manifesting as cardiovascular collapse, bronchospasm, angioedema, urticaria, and erythema; infusion reactions; cytokine release syndrome; autoimmune reactions; cytotoxic type II and immune complex type III hypersensitivities; and delayed cell-mediated type IV hypersensitivities often manifesting as cutaneous reactions [202]. In February 2013, the FDA issued a draft guidance entitled 'immunogenicity assessment for therapeutic protein products' which, it was stated, would, in its final form, represent the FDA's thinking on the topic [203]. The guidelines basically represent a risk-based approach to evaluating and mitigating immune responses to therapeutic proteins that might reduce, change, or eliminate the intended therapeutic action and/or induce adverse events otherwise not seen in the absence of an antibody- or cellular-targeted response. The immunogenicity of an administered protein such as the fusion proteins discussed here can be affected by both patient-specific and product-specific factors [203]. Patient-related factors include: (1) The immunological status and competence of the patient, which is relevant to, for example, the patient's age and whether the patient is immune suppressed or immune activated (as in infection); (2) Prior sensitization and/or a history of allergy; (3) The route, dose, frequency, and length of administration; (4) The genetic status of the patient, e.g., some HLA haplotypes may predispose patients to an adverse event; (5) The patient's status of immune tolerance to endogenous proteins, e.g., the presence of autoantibodies to cytokines and growth factors and the possibility that a recombinant therapeutic protein might induce, or break, tolerance to an endogenous protein. Some important product-specific factors include: (1) The product's origin—non-human protein always has the potential to be immunogenic; (2) Primary molecular structure and

post translational modifications—the primary sequence is especially important for fusion proteins where a potential exists for new antigens to form from the linking of foreign and endogenous proteins; (3) Quaternary structure: protein aggregates—Fc-fusion proteins are well known to aggregate and misfold and disulfides may not pair as desired; (4) Pegylation (see earlier) and glycosylation—clearance may be accelerated, due, for example, to incomplete glycan chains with terminal D-galactose or *N*-acetyl-D-glucosamine; (5) Immunomodulatory properties of the protein, e.g., IL-2 is both immunogenic but also up-regulates immune responses to endogenous proteins and may induce clinical autoimmunity.

Because interactions between the Fc domain and its receptors have immunologic consequences, attention has been drawn to Fc fusion as a platform technology for modulating immunogenicity. It has been claimed that while administration of a suitably engineered Fc fusion partner may improve both the disease outcome and the safety profile of the fusion partner, interactions between the Fc domain and its receptors raise concerns about the long-term use of Fc-fusion proteins [204].

The higher incidence of reactions to the anti-TNF mAb infliximab in patients with rheumatoid arthritis compared with patients with ankylosing spondyloarthritis and vasculitis demonstrates that the disease itself can also be important in the development of an immune response to a biological agent [205]. From this brief consideration of the complexities of immunogenicity of protein biologics, it is not hard to see that, at the moment, the risk of an immune response to a fusion protein cannot be estimated or eliminated. The risk can, however, be managed.

5 Diagnosis of Hypersensitivities to Fusion Proteins, Premedication, and Desensitization

Apart from a still small but growing body of investigation on a few of the mAbs approved for human therapy [202, 206, 207], few data are available on appropriate diagnostic and desensitization procedures for the investigation of hypersensitivity responses to the steadily increasing number of approved cytokines, enzymes, toxins, and chimeric fusion proteins. What information there is on the fusion proteins tends to be limited almost exclusively to etanercept. For the diagnosis of immediate and delayed hypersensitivities to fusion proteins, no tests, validated or otherwise, for the detection of specific IgE antibodies are generally available leaving skin testing, as yet unstandardized, as the prime diagnostic procedure for detecting IgE-mediated reactions and T-cell-mediated adverse events. For prick testing, concentrations of 5–25 mg/mL of etanercept have been used [130–132, 208]; for intradermal testing, 0.0025–0.25 mg/mL [132, 208] and 0.1–5 mg/mL [130, 131]. Patch testing has been undertaken with etanercept at concentrations of 1 and 5 % in petrolatum [132]. It is clear that many reactions induced by individual fusion proteins remain inadequately investigated and given their chimeric nature, the list of hypersensitivity reactions they provoke is likely to expand. Diagnostic recommendations have been made for immune thrombocytopenia and although immunoassays to detect platelet-reactive antibodies are available in a few laboratories, the tests are not standardized, do not detect the drug-dependent antibodies, are technically difficult, and may produce false positives. The situation is similar for other type II hypersensitivities such as immune neutropenia where the monoclonal antibody immobilization of granulocyte antigens assay seems to be the test of choice [209]. Diagnostic shortcomings, owing in some cases to lack of laboratory markers and absence of routine tests for type III hypersensitivity immune complex reactions such as serum sickness and vasculitis, extend to some fusion protein-induced liver and lung injuries and delayed cutaneous reactions (see [210] for discussion and further references). The pathomechanisms underlying some delayed skin reactions to fusion proteins have been little studied. Apart from what appears to be a few cell-mediated, type IV true hypersensitivities [69–81, 201, 211], some fusion protein-induced skin responses may represent direct targeting events that are not genuine hypersensitivities and are similar to, for example, agents that bind epidermal growth factor receptor causing non-immune-mediated adverse cutaneous events [202, 210, 212] or biologics that provoke exacerbation of psoriasis.

Premedication along with infusion rate adjustment may be used to reduce the incidence of adverse reactions to biological agents, although opinion on the effectiveness of the former is divided. For mAbs such as rituximab, acetaminophen and anti-histamines are often given [207]. A commonly used premedication protocol employs a corticosteroid, usually dexamethasone or prednisolone, and H_1 and H_2 antihistamines such as diphenhydramine and cimetidine/ranitidine/famotidine are often administered 12 and 6 h before infusion [202]. Corticosteroids are sometimes given for several days before infusion [202]. There are a few reports of successful desensitization of acute injection-site reactions to etanercept. After the finding of a positive skin test in a patient who developed pruritus, redness and swelling following the 22nd injection of etanercept and a strong positive challenge to the fusion protein that persisted for a month, a 4-day desensitization regimen was undertaken [208]. One hour prior to the commencement of the desensitization procedure, the patient who was on daily cetirizine throughout, was given oral aspirin 325 mg, montelukast 10 mg, diphenhydramine 25 mg, and famotidine 40 mg. On days 1 and 2, a starting

dose of 0.25 mg was given and this was built up slowly at regular intervals until a total dose of 12.5 mg was administered. On day 3, a similar schedule starting with 0.5 mg was increased at intervals up to a maximum of 24.75 mg of etanercept. On day 4, a total of 25 mg of the protein was administered in a single dose. The patient was maintained on twice-weekly etanercept injections with diphenhydramine 25 mg 1 h before each injection and daily cetirizine. A closely similar protocol was used successfully in another patient with a severe injection-site reaction to etanercept [213]. Two further cases of successful desensitization in etanercept-sensitive patients have been reported. A patient with a progressively severe injection-site reaction who developed drug-induced lupus was given etanercept by incremental subcutaneous injections in the range of 0.025–12.5 mg every 30 min on day 1 and started on 7.5 mg twice weekly on day 3. A second patient also with a severe injection-site reaction to etanercept received four incremental subcutaneous injections in the range of 0.25–5 mg every 30 min on day 1, 7.5, 10, and 15 mg each 30 min apart on day 3 and 20 mg on day 5. From day 8 the patient was maintained symptom free on 12.5 mg twice weekly [214]. In what was claimed as a novel method of "desensitization", immunosuppressant therapy with methylprednisolone together with methotrexate (7.5 mg) given together as a single dose was used successfully to treat an etanercept-induced urticarial eruption. One week later when the patient's condition had markedly improved, a second dose of the two-drug combination was administered. After a further week, the urticaria had completely abated and thereafter etanercept was continued at 50 mg a week [215].

Following the successful desensitization of two patients who experienced an injection-site reaction to etanercept [208], the same investigators standardized their methodology in successfully desensitizing a further seven patients, six of whom had an injection-site reaction to etanercept and one with an immediate systemic reaction [216]. Each of the injection-site reactions consisted of local pruritus, erythema, and swelling or edema. The immediate reaction included swollen face and lips, urticaria, wheezing, dyspnea, nausea, vomiting, and hypotension. Injection-site reactions are thought to be T-cell-mediated, delayed type IV hypersensitivities but sometimes involving, or developing into, an IgE-mediated immediate reaction [57, 131], making continued therapy with any provoking drug problematic. All seven patients were skin test positive to etanercept, the six with injection-site reactions responding to intradermal test concentrations of 0.025–0.5 mg/mL. The desensitization protocol, described as rapid, was carried out over 3 days with the administration of six subcutaneous injections (0.5, 1, 2, 4, 8, 9 mg etanercept) at 30-min intervals for a total of 24.5 mg on days 1 and 2. On day 3, seven doses (0.5, 1, 2, 4, 8, 16, 18.5 mg) for a total of 50 mg were given at 30-min intervals. A maintenance weekly injection of etanercept with cetirizine premedication enabled all seven patients to continue with etanercept therapy. Minor local erythema resolved within 1–2 h.

6 Conclusions

Realization that the interaction of human IgG with the salvage neonatal FcRn receptor can be exploited to prepare and deliver therapeutically useful proteins in chimeric form, has seen Fc fusion proteins become increasingly recognized as a valuable and safe form of biologics therapy. Advances in knowledge and application of molecular cloning, antibody engineering techniques, protein design, development of cell lines, and bioprocessing have all contributed to the general strategy of fusing selected effector domains of short-lived but otherwise therapeutically promising macromolecules with a suitable fusion partner, often the Fc fragment of human IgG but sometimes human serum albumin or transferrin. In fact, because many peptides and proteins have short half-lives owing to rapid renal clearance, of the several beneficial biological and pharmacological properties exhibited by such chimeric fusion proteins, the prime stimulus for their development is usually plasma therapeutic half-life extension. Other benefits of IgG Fc fusion include the capacity of the attached Fc domain to recognize Fc receptors on immune cells (potentially important for vaccines and cancer therapy); the conferring of immunomodulatory properties such as the induction of tolerance to immunogenic effector proteins; improved solubility and stability of proteins; and facilitation of the manufacturing process by protein A affinity purification [7]. Looking at the efficacy and safety of the fusion proteins already approved; the promising clinical pipeline of candidates [4, 7]; the impending appearance of biosimilars [15]; the range of potential effector proteins that can be used as fusion partners; the promise of Fc fusion proteins as vaccines and as therapies for a much wider range of disorders [11]; and the commercial success of the first generation of the chimeric macromolecules [4, 5], subsequent generations of therapeutic fusion proteins are anticipated with considerable optimism.

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Since this review entered the production stage, four more fusion proteins, albiglutide (fused to albumin) and dulaglutide (an Fc fusion), both for glycemic control, and Fc fusion proteins of coagulation factors VIII and IX have been approved by the FDA.

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