

Biological Therapeutics and Pharmacovigilance in Italy

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Abstract: Biological Therapeutics (BTs) present a novel frontier for the treatment of autoimmune diseases, such as rheumatoid arthritis, psoriasis, Crohn's disease and several other conditions. BTs constitute highly selective compounds targeted upon specific structures that may be proteins, receptors, or DNA sequences. In the case of autoimmune diseases, the use of BTs is directed against pro-inflammatory cytokines that exert a central role in the inflammatory machinery. In the present review, attention is focused upon BTs that inhibit pro-inflammatory cytokines thereby blocking the inflammation, such as monoclonal antibodies (e.g. infliximab and adalimumab) and soluble receptors (e.g. etanercept). The interleukin-1 and interleukin-6 antagonists, anakinra and tocilizumab, rituximab, which decrease the number of circulating B-lymphocytes and abatacept, thereby counteracting T-lymphocyte activation, are described also. Despite the utility

of BTs for patients presenting autoimmune diseases, they have been linked to opportunistic viral, bacterial, mycotic infections and to tumor cases. The occurrence of these pathologies is due to their immunosuppressive functions thereby requiring the meticulous monitoring by pharmacovigilance and drug safety techniques to assess risk analysis. Whether or not Adverse Drug Events (ADEs) occur more frequently in patients administered BTs, compared to traditional drugs, is currently an essential topic of investigation.

Keywords: BTs; Cytokines; Infliximab; Adalimumab; Etanercept; Anakinra; Tocilizumab; Adverse drug reactions; Pharmacovigilance; Adverse drug effects;

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Introduction

Biological therapeutics (BTs), on the frontier of pharmacology and biotechnology, concerns highly selective compounds targeting specific structures (for example a protein, a receptor, a DNA sequence) involved in autoimmune disease pathogenesis, thereby reducing collateral effects and increasing the efficacy of medical therapies. Currently, BTs for the treatment of autoimmune diseases, such as rheumatoid arthritis, psoriasis, Crohn's disease (CD) and for some tumors are already available [1-3]. These practical advances have been due to important discoveries in the fields of cytokine biology and monoclonal antibody development [4]. The use of BTs in autoimmune disease is directed against pro-inflammatory cytokines that exert a central role in the inflammatory machinery. These agents may: (i) deter these cytokines thereby blocking inflammation through an action that antagonizes tumor necrosis factor- α (TNF- α), e.g. monoclonal antibodies (infliximab and adalimumab) and soluble receptors (etanercept), (ii) antagonize interleukin-1 (IL-1), e.g. anakinra, and interleukin-6 (IL-6), e.g. tocilizumab, and (iii) reduce the number of circulating B-lymphocytes (rituximab) or T-lymphocyte activation (abatacept). Tumor therapies utilize monoclonal antibodies acting on growth factors involved in tumor proliferation. These monoclonal antibodies may be grouped into three different classes: (a) antibodies against the Epidermal Growth Factor (HER-1), such as cetuximab, panitumumab, erlotinib, (b) antibodies against Vascular Endothelial Growth Factor (VEGF) and against Epidermal Growth Factor (HER-2), bevacizumab and trastuzumab and (c) "multi target antibodies" acting at different levels such as lapatinib, sunitinib e sorafenib. Bi or multi-specific antibodies are capable of blocking multiple growth and survival pathways and thereby able to block cancer growth. Indeed,

many of these antibodies are being advanced in clinical development [5, 6].

In the present review, attention is focused upon the compounds exerting an anti-TNF- α action, infliximab, adalimumab and etanercept, on those therapeutics that antagonize IL-1 (anakinra) and IL-6 (tocilizumab), and on those compounds which act to reduce the number of circulating B-lymphocytes (rituximab) or T-lymphocytes activation (abatacept).

Anti-TNF- α Biological Therapeutics (Infliximab, Etanercept and Adalimumab):

TNF- α is a naturally occurring cytokine that is involved in normal inflammatory and immune responses. Elevated levels of TNF- α are found in the tissues and fluids of patients presenting rheumatoid arthritis (RA), psoriatic arthritis, ankylosing spondylitis (AS), and plaque psoriasis. There are two distinct receptors for TNF (TNFRs): a 55 kilodalton protein (p55) and a 75 kilodalton protein (p75). The biological activity of TNF- α is dependent upon binding to either cell surface receptor (p75 or p55). High levels of TNF- α have been associated with the development of intestinal inflammation in CD, a chronic inflammatory disease of the intestines that frequently occurs in the lower part of the small intestine (the ileum), but which may affect any part of the digestive tract. The most common symptoms of CD include abdominal pain, often in the lower right region of the abdomen, and diarrhea. In CD, many cytokines are elevated, such as the type 1 (Th1) cytokines which include TNF- α , interleukin-2 (IL-2), and interferon- α [7]. TNF- α is an adipokine, a pleiotropic inflammatory cytokine secreted by adipose tissue, but produced also by lymphocytes and macrophages, and involved in the acute phase reaction; it is a trimeric protein encoded within the

major histocompatibility complex and may induce fever, apoptotic cell death, cachexia, and inhibit tumorigenesis (or carcinogenesis). Dysregulation of the cytokine balance is implicated in Alzheimer's disease [8-10] autism [11] clinical depression [12] cancer [13] and inflammatory bowel disease [14]. BTs offer interventions that differ from traditional pharmacological therapies. TNF- α blocking agents (infliximab, adalimumab) bind to TNF- α molecule thereby neutralizing their biological activity resulting in the healing of intestinal inflammation. All four molecules are synthetic antibodies which is based upon TNF- α binding [15]. TNF- α inactivation is essential for the down-regulation of inflammatory reactions that are associated with autoimmune diseases (Figure 1; Tables 1, 2)

Infliximab, a monoclonal antibody and TNF- α antagonist [16] is an artificial mouse-human chimeric antibody to TNF- α developed in the mouse

using standard and modified techniques. Antigenicity is reduced through the substitution of the constant chain of the mouse antibody with the human IgG1 constant chain. Infliximab has been administered also in the treatment of other disease conditions, such as **psoriasis** and ankylosing spondylitis [17, 18].

Other BTs and monoclonal antibodies have not showed as much efficacy in the treatment of

intestinal bowel diseases (IBD). These include **etanercept**, which is the soluble receptor for TNF- α [19]. **Adalimumab**, which is a humanized **recombinant** antibody to TNF- α , showed efficacy in patients with moderate-to-severe CD, but less than that of infliximab [20]. Adalimumab nevertheless, has the advantage of administration through subcutaneous injection as opposed to infliximab, which is administered through intravenous infusion (Figure 1A).

Figure 1 A



Etanercept is a soluble receptor, a “decoy receptor”, which binds specifically to TNF- α and blocks its interaction with cell surface receptors thereby providing a TNF inhibitor action. It is a

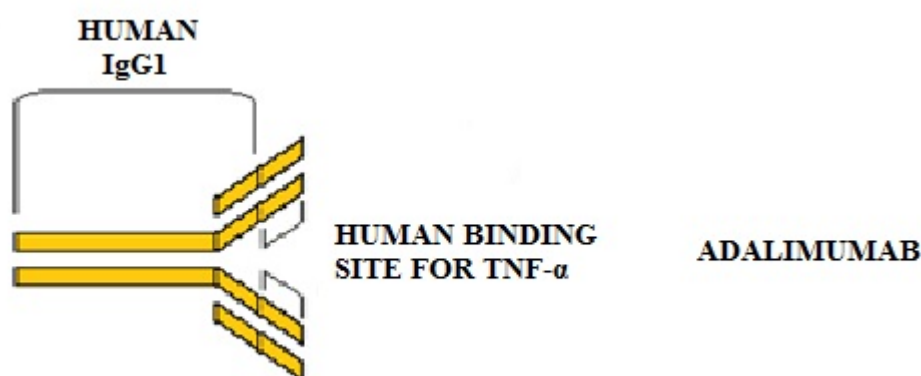
dimeric molecule, a soluble form of the p75 TNF receptor that can bind to two TNF molecules, thereby effectively removing them from circulation. It consists of the extracellular ligand-binding portion

of the human 75 kilodalton (p75) TNF receptor (TNFR) linked to the Fc portion of human IgG1. The Fc component of etanercept contains the CH2 domain, the CH3 domain and hinge region, but not the CH1 domain of IgG1. Its anti-inflammatory action is especially useful in autoimmune diseases.

Binding assays using radioactively labeled TNF- α have revealed that infliximab binds both monomeric (inactive) and trimeric (active) forms of soluble TNF whereas etanercept is more likely to bind the active

trimeric form. Furthermore, infliximab forms stable complexes with soluble TNF- α whereas etanercept tends to form relatively unstable complexes, allowing dissociation of TNF- α and the potential formation of a reservoir for binding TNF- α . Etanercept alone is able to bind to and neutralize lymphotoxin (TNF- β), produced by T-cells inducing vascular endothelial cells to change their surface adhesion molecules to allow phagocytic cells to bind to them [21] (Figure 1B).

Figure 1 B



Adalimumab is capable of reducing pain and flogosis (inflammation with swelling) in psoriatic and RA in which a cytokine cascade leads to inflammation. Like infliximab and etanercept, adalimumab binds to TFN- α , preventing it from activating TNF receptors. In RA, activated T cells invade the synovial membrane and release cytokines including IL-2, interferon (IFN)- α , TNF- α and IL-3. This causes synovial macrophages and fibroblasts to produce the inflammatory cytokines TNF- α , IL-1 and IL-6, which lead to complex signal-transduction cascades and to the production of molecules

involved in tissue degradation which results in the resorption, uptake from tissue to blood, and destruction of bone tissue. Adalimumab is produced by phage-display technology, resulting in an antibody with human-derived heavy- and light-chain variable regions, and human immunoglobulin (Ig) G1 constant regions [22].

Adalimumab represents the third TNF inhibitor, after infliximab and etanercept, to be approved in the United States. Like infliximab and etanercept, adalimumab binds to TNF- α , preventing it from activating TNF receptors. Adalimumab was

constructed from a fully human monoclonal antibody. The anti-TNF- α is associated with autoimmune disease alleviation. Adalimumab is applied for the treatment of RA, psoriatic arthritis, ankylosing spondylitis, CD, moderate to severe chronic, psoriasis, juvenile idiopathic arthritis (JIA), and ulcerative colitis. It has been found to be useful in cases that have not responded to conventional treatment at standard dosing for CD [23,24].

However, because TNF- α is part of the immune system that protects the body from infection, prolonged treatment with adalimumab may increase the risk of developing infections [25-28]. In patients presenting systemic vasculitis, the anti-TNF- α agents, infliximab, etanercept, tocilizumab and adalimumab, were shown to be efficacious [29,30], with some degree of corticosterone-sparing (Figure. 1C)

Figure 1 C

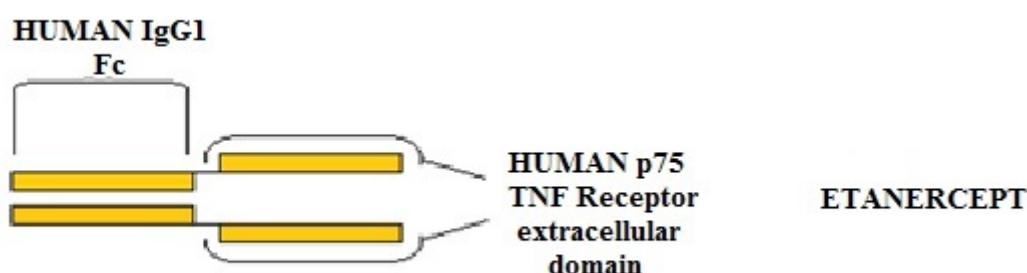


Figure 1: Mechanism of action of the anti-TNF- α agents, infliximab, etanercept and adalimumab (see text). (A) Infliximab is a monoclonal artificial mouse-human chimeric antibody to TNF- α , in which the constant chain of the mouse antibody is substituted with the human IgG1 constant chain. (B) Etanercept consists of the extracellular ligand-binding portion of

the human p75 TNFR linked to the Fc portion of human IgG1. The Fc component of etanercept contains the CH2 domain, the CH3 domain and hinge region, but not the CH1 domain of IgG1. (C) Adalimumab is an antibody constituted by the human-derived heavy- and light-chain variable regions, linked to the human IgG1 constant regions

Table 1. BTs administered for the treatment of autoimmune diseases: mechanism of action, type of molecule and compound

Mechanism of Action	Molecule type	Compound
TNF- α antagonist	monoclonal antibody	infliximab, adalimumab
TNF- β antagonist	soluble receptor for TNF- β	Etanercept
Mechanism of Action	Molecule type	Compound
IL-1 antagonist	competitively inhibits the binding of IL-1 to the interleukin-1 type 1 receptor	Anakinra
IL-6 antagonist	Humanized monoclonal antibody against the interleukin-6 receptor (IL-6R)	Tocilizumab
Mechanism of Action	Molecule type	Compound
Circulating B lymphocytes number reducer	human/murine chimeric monoclonal antibody directed against the CD20 protein that is found both on normal and leukemic lymphocytes	Rituximab
T-lymphocytes activation reducer	fusion protein composed of the Fc region of the immunoglobulin IgG1 fused to the extracellular domain of CTLA-4. It is a molecule capable of binding with more avidity to CD80 (B7-1) than to CD86 (B7-2). Abatacept is a selective co-stimulation modulator as it inhibits the costimulation of T cells	Abatacept

Table 2. Compounds available for the treatment of autoimmune pathologies and the related disease states.

Compound	Pathologies
infliximab, adalimumab, etanercept	rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis (AS), plaque psoriasis, Crohn's disease, JIA ulcerative colitis (Behm et al 2009; Sandborn et al 2001; Moses J. et al. 2012; Griffin et al 2000; Hanauer et al 2006; Bain and Brazil 2003; Burmester et al 2013; Ungar et al 2013)
Anakinra	rheumatoid arthritis also applied in combination with methotrexate, JIA, psoriatic arthritis, spondylarthritis, Still's Disease, destructive osteoarthritis in inflammatory phases, type 2 diabetes (Cohen et al., 2002; Waugh and Perry 2005; Jung et al., 2010; Kiltz et al., 2012; Pascual et al., 2005; Illiou et al., 2013; Ungar et al., 2013; Iqbal and Fleischman, 2007; Larsen et al., 2007).
tocilizumab	treatment of moderate to severe rheumatoid arthritis, applied in combination with methotrexate; JIA, tocilizumab is combined with methotrexate unless the latter is not tolerated Hoffmann-La Roche 2009; Fleischmann et al. 2013; De Benedetti et al 2013; Horneff 2013). In Japan, tocilizumab is also approved for the treatment of Castleman's disease , a rare benign tumor of B cells (Matsuyama et al., 2007; "Assessment report for RoActemra". ("Assessment report for RoActemra".)
rituximab	treatment of autoimmune diseases such as vasculopathies, multiple sclerosis, diabetes, lupus erythematosus JIA and also for B cell non Hodgkin lymphoma and leukemia (Gurcan et al 2009; Boross and Leusen 2012; Czuczman et al 2005; Ungar et al 2013)
abatacept	rheumatoid arthritis, JIA (Ungar et al., 2013; Keating, 2013).

Biological Therapeutics Reducing the Number of Circulating B-lymphocytes (rituximab) and Activated Tlymphocytes (abatacept)

Rituximab is administered for the treatment of autoimmune diseases such as vasculopathies, multiple sclerosis, diabetes, lupus erythematosus and also for B cell non Hodgkin lymphoma and leukemia [31]. It is a human/murine chimeric monoclonal antibody directed against the CD20 protein that is found both on normal and leukemic lymphocytes. The CD20 protein is a differentiation cluster expressed on the surface of immune system B cells from early differentiation stages onwards, but is absent on final differentiation plasma cell stages. It is not known how the CD20 complex acts but may be involved in the Ca^{2+} flow through the cell membrane thereby regulating Ca^{2+} internal cell concentration with eventual B cell activation [32]. Rituximab is involved in the destruction of B cells thereby with utility for treatment of diseases characterized by excessive numbers of B cells, overactive B cells, or dysfunctional B cells. It induces the immune system to target and destroy the cells that it adheres to. Normal lymphocytes then grow to replace those that have been destroyed. Nevertheless, rituximab acts against all of the B lymphocytes thereby abolishing both normal and cancer cells; for this reason, it is used for the treatment of diseases characterized by a high number of hyperactive or dysfunctional B cells.

Rituximab, in combination with the chemotherapy drugs, fludarabine and cyclophosphamide, is the most commonly used first line treatment for individuals presenting chronic lymphocyte leukemia (CLL) [32,33] (Figure 2A; Tables1, 2).

Abatacept is a fusion protein composed of the Fc region of the immunoglobulin IgG1 with the hinge,

CH2, and CH3 domains fused to the extracellular domain of Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) [34]. CTLA-4, also known as CD152 (Cluster of differentiation 152), is a protein receptor that down-regulates the immune response. CTLA4 is found on the surface of T cells. T cell activation can be activated by stimulating the CD28 receptor on the T cell and deactivated by stimulating the CTLA4 receptor, which acts as an "off" switch [35].

Under normal conditions, full T cell activation requires: 1) binding of the T cell receptor to the antigen-MHC complex on the APC (Antigen Presenting Cells) and 2), a co-stimulatory signal provided by the binding of CD28 to the B7 protein on the APC. B7-1 /CD80 (Cluster of Differentiation 80) is a protein found on activated B cells and monocytes that provides a co-stimulatory signal necessary for T cell activation and survival. It is the ligand for two different proteins on the T cell surface: CD28 and CTLA-4 [36, 37]. CD80 works in tandem with CD86 to prime T cells. Abatacept, which contains a high-affinity binding site for B7, that is CTLA-4, binds to the B7 protein on APCs thereby preventing them from delivering the positive co-stimulatory signal to T cells, that is the CD28 binding, thus preventing the full activation of T cells [38,39]. Abatacept is a molecule capable of binding with more avidity to CD80 (B7-1) than to CD86 (B7-2). It was developed by Bristol-Myers Squibb and is licensed in the United States for the treatment of RA in the case of inadequate response to anti-TNF- α therapy. It delays the progression of structural damage and reduces symptoms of RA. However, it should not be used in combination with anakinra or TNF- α antagonists. Abatacept, together with other agents, such as infliximab, adalimumab, anakinra and rituximab, is used for the treatment of JIA for those patients that give an inadequate response to conventional treatments.

Abatacept offers the basis for the belatacept second-generation currently being tested in clinical trials. It is a selective co-stimulation modulator as it inhibits the co-stimulation of T-cells. The two compounds differ by only 2 amino acids. In organ

transplantation, belatacept is intended to provide extended graft survival while limiting the toxicity generated by standard immune-suppressing regimens such as calcineurin inhibitors (for example cyclosporin) [40, 41] (Figure 2B; Tables 1, 2)

Figure 2

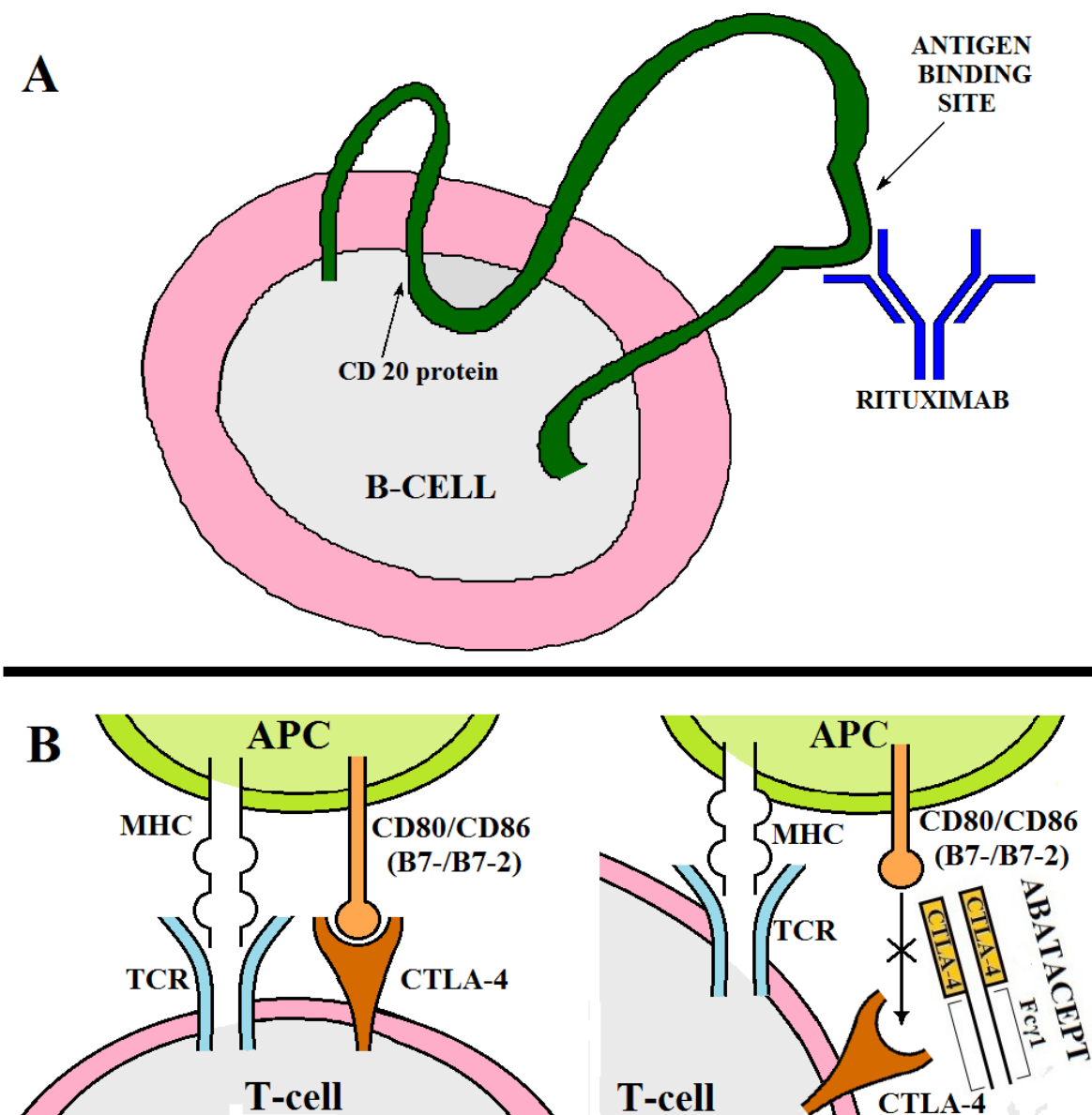


Figure 2: Mechanism of action of agents which reduce the number of circulating B lymphocytes (rituximab) or of activated T-lymphocytes (abatacept) (see text). (A) Rituximab is a human/murine chimeric monoclonal antibody directed against the CD20 protein that is found both on normal and leukemic lymphocytes from early differentiation stages onward, but it is absent on final differentiation stages. Rituximab, linking to CD20 protein, induces the immune system to target and destroy the cells that it adheres to. (B) Abatacept is a fusion protein composed of the Fc- γ 1 with the hinge, CH2, and CH3 domains fused to the extracellular domain of CTLA-4. CTLA-4 is found on the surface of T cells. T cell activation requires the binding of TCR to the antigen-MHC complex on the APC and a co-stimulatory signal provided by the binding of CTLA-4 to CD80/CD86 (left panel). Abatacept acts by inhibiting the CTLA-4 CD80/CD86 binding (right panel).

Interleukin-1 (IL-1) and Interleukin-6 IL-6 Antagonists

Anakinra (Figure 3, Tables 1, 2) molecule is a recombinant, non-glycosylated version of human IL-1 receptor Antagonist (IL-1RA) prepared from cultures of genetically modified *Escherichia coli* (*E. coli*) using recombinant DNA technology. It consists of 153 amino acids and has a molecular weight of 17,257.6 g/mol (approx. 17.3 kilodaltons) and differs from native human IL-1RA in that it has the addition of a single methionine residue on its amino terminus [42-44].

Anakinra blocks the biologic activity of naturally occurring IL-1, involving inflammation and cartilage degradation associated with RA, by competitively inhibiting the binding of IL-1 to the IL-1 type 1 receptor, which is expressed in many tissues and organs. IL-1 is produced in response to

inflammatory stimuli and mediates various physiologic responses, including inflammatory and immunologic reactions. Additionally, it stimulates bone resorption and induces tissue damage, like cartilage degradation as a result of loss of proteoglycans. In patients with RA, the natural IL-1 receptor antagonist is not found in effective concentrations in the synovium and synovial fluid in order to counteract the elevated IL-1 concentrations. Anakinra may be administered in combination with methotrexate [45].

Anakinra is not considered a 'Disease-modifying antirheumatic drug' (DMARD) but rather a 'Biological Response Modifier' (BRM) because it is able to selectively target the pathologic element of the disease. It has a bioavailability of 95% for healthy adults after a 70 mg subcutaneous bolus injection. Peak plasma concentrations of anakinra generally occur 3 to 7 hours after administration of clinically relevant doses (1 to 2 mg/kg) for patients with RA. The terminal half-life ranges from 4 to 6 hours. After daily dosing for up to 24 weeks, no unexpected anakinra accumulation is observed in the plasma samples of RA patients [46].

Anakinra may be effective in pediatric patients presenting JIA (also known as juvenile rheumatoid arthritis, JRA), or Still's Disease [47]. Much accumulating evidence supports the utilization of anakinra in Adult-Onset Still's Disease [48, 49]. Due to the specific mechanism of action of anakinra, it has been applied effectively in patients presenting inflammatory joint diseases, such as psoriatic arthritis, and spondylarthritis [50,51]. Possibly, anakinra may even benefit patients presenting destructiveosteoarthritis in inflammatory phases[52]. The compound may be useful for the treatment of type 2 diabetes too [53]. Since Anakinra (IL-1ra) is efficiently used in the clinical treatment of autoinflammatory syndromes, as well as for gout

patients, the potential use of Anakinra in order to retard the progression of asbestosis, silicosis and possibly other inflammatory lung diseases has been considered. It appears likely that IL-1 inhibitors may be effective in gouty arthritis, [54] and in the treatment of colchicine-resistant Familial Mediterranean Fever [55]. The effectiveness of

Anakinra was demonstrated in stroke with MRI scans revealing that the rats that administered the compound up to three hours after the stroke, presented only about half the brain damage of the placebo group [56]. It terminated also epileptic seizures rapidly, prevented recurrence and repaired seizure-associated BBB breakdown [57].

Figure 3

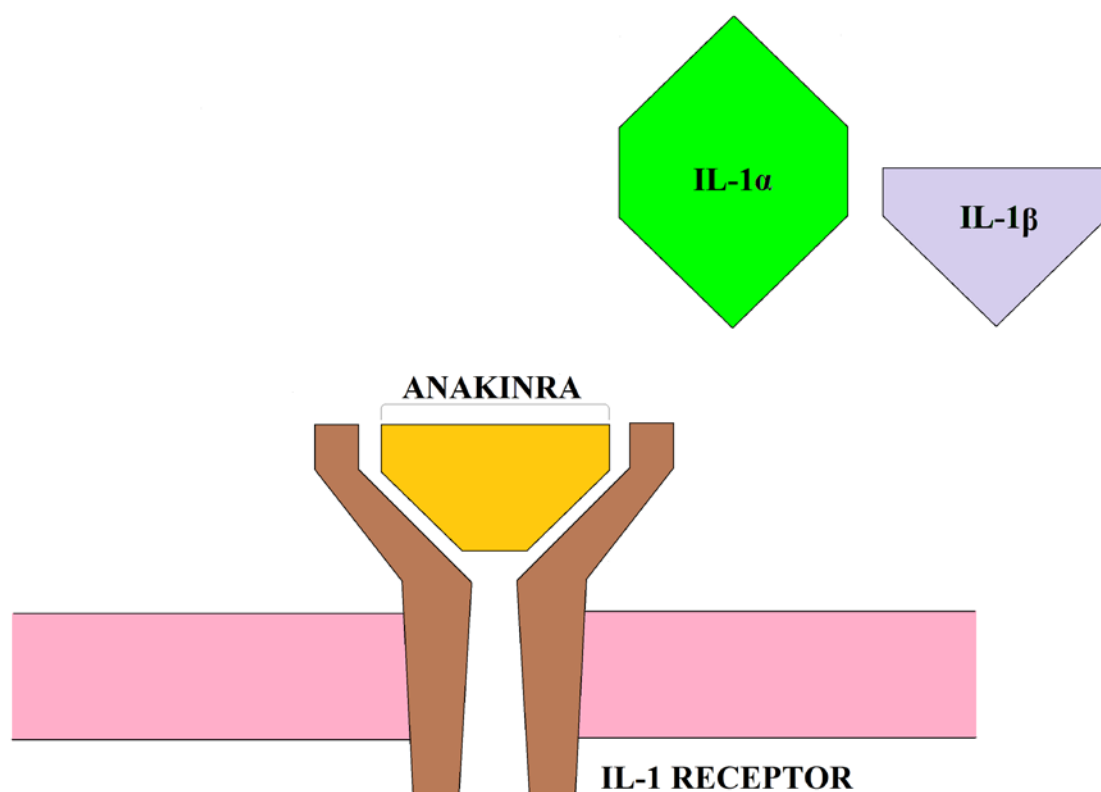


Figure 3: Mechanism of action of the anti-interleukin-1 agent, anakinra (see text). Anakinra competitively inhibits the binding of IL-1 α and of IL-1 β to their receptor (IL-1 type 1 receptor).

Tocilizumab (Figure 4, Tables 1, 2) is a humanized monoclonal antibody against the interleukin-6 receptor (IL-6R) that binds soluble as well as membrane bound IL-6 receptors, hindering IL-6, that is implicated in autoimmune diseases, multiple

myeloma and prostate cancer [58], from exerting its pro-inflammatory effects. IL-6 is present at elevated levels in RA-affected patients and in subjects suffering from systemic JIA, a severe form of RA in children. Autoimmune diseases like RA are

associated with abnormally high IL-6 levels. The drug is administered by monthly intravenous infusions. An infusion takes about an hour [59-61].

Tocilizumab is used for the treatment of moderate to severe RA, applied in combination with methotrexate, used in cancer therapy, if other drugs like “disease-modifying antirheumatic drugs” (DMARDs) and TNF- α blockers have proven to be ineffective, or were not tolerated. It can be used as a single medication for patients who do not tolerate

methotrexate (Reference note 3; "Assessment report for RoActemra". European Medicines Agency). The drug retards disease progression and may improve physical function of patients [62]. The treatment of systemic JIA is similar to RA treatment: tocilizumab is combined with methotrexate unless the latter is not tolerated. General safety and efficacy is established for children of two years and older [63]. In Japan, tocilizumab is approved also for the treatment of Castleman's disease, a rare benign tumor of B cells [64, 65].

Figure 4

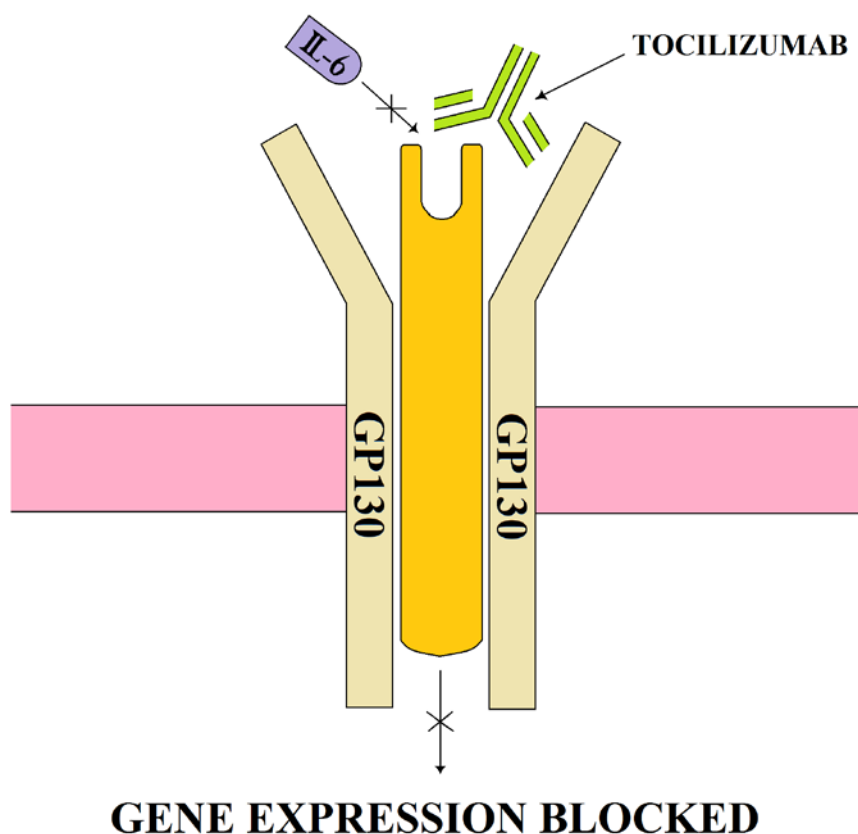


Figure 4: Mechanism of action of the anti-interleukin-6 agent, tocilizumab (see text). Tocilizumab is a humanized monoclonal antibody directed against the interleukin-6 receptor (IL-6R). Tocilizumab links both soluble and membrane bound receptors, thus hindering the linkage of IL-6 to its receptors.

Adverse Drug Effects (ADEs)

BTs offer as a key option for patients presenting autoimmune diseases and yet have been associated with opportunistic viral, bacterial and mycotic infections. The containment of this type of infection hinges upon the immunosuppressive (reduction of activation/efficacy of the immune system) properties of these therapeutic agents; yet, immunosuppression may occur as an adverse reaction to treatment of other conditions. A common ADE of immunosuppressive compounds is immunodeficiency, due to non-selective action that induces elevated susceptibility to infection and reduced cancer immunosurveillance. Other ADEs include hypertension, dyslipidemia, hyperglycemia, peptic ulcers, lipodystrophy, moon face, and liver and kidney damage.

Cases of tuberculosis, listeriosis, pneumocytosis, pneumonia, septicemia, septic arthritis, and systemic mycoses are caused by opportunistic pathogens that infect patients with immune deficiencies who would otherwise not be infected, and conditions such as histoplasmosis, coccidiomycosis, blastomycosis, AIDS and metastatic cancer have been reported [66-71]. A compromised immune system creates an 'opportunity' for these agents, bacterial, viral, fungal or protozoan infections that usually do not cause disease in a healthy host, one with a healthy immune system, for pathogens to infect the individual. It is clear that the administration of TNF- α inhibitors demands a careful analysis of the risk, above all in patients showing a positive anamnesis for recurrent infections, general physical conditions that may induce the development of infections or for subjects residing in areas endemic for serious infections. During the course of TNF- α inhibitor therapy, patients must be monitored for the risk of developing opportunistic infections.

The US food and drug administration (FDA) has provided health risk warnings concerning the risk of infection from two bacterial pathogens, *Legionella* and *Listeria*. People taking TNF blockers are at increased risk for developing serious infections that may lead to acute hospitalization or death due to bacterial, mycobacterial, fungal, viral, parasitic, and other opportunistic pathogens [72]. Latent tuberculosis infection reactivation present one of the major complications linked to TNF- α inhibitor treatments [73], with dose-dependent episodes associated with the therapy independent of administration regime. Furthermore, it seems that the tuberculosis risk is higher for anti-TNF- α monoclonal antibody therapy rather than for soluble TNF-receptor therapy [73], with higher frequency adalimumab and infliximab compared to etanercept [73]. It ought to be noted that the current use of anti-TNF- α agents in patients presenting hepatitis B virus (HBV) may instigate a viral reactivation. The percentage of reactivation is higher in patients previously treated with immunosuppressive agents (96% vs. 70%, $p=0.033$) and lower in those who received antiviral prophylaxis (23% vs. 62%, $p=0.003$). Acute liver failure and death has also been reported among HBV positive patients. Infliximab was associated with a higher rate of induced liver disease (raised transaminase levels, clinical signs, viral reactivation, and acute liver failure) compared with etanercept. The increasing number of reported cases of HBV reactivation following TNF-targeted therapies and the associated morbidity and mortality demand specific preventive strategies.

Malignant neoplasia incidence, above all lymphoma, appears to be higher in patients treated with TNF- α inhibitors when compared to control groups. A higher number of leukemia cases have been observed in patients treated with anti TNF- α therapeutic agents, too. In patients affected with RA,

an increased incidence of lymphomas and leukemia has been observed. Fatal tumors have been reported among children and young adults treated with anti TNF- α therapeutics (<23 years old); half of these tumors were lymphomas. On the basis of literature data and indications, the FDA has started a safety analysis for tumor risk in patients treated with TNF- α inhibitors aged 30 or younger [Reference note 2].

Anti TNF- α agents induce adverse complications due to immunogenicity, the propensity of particular substances, e.g. antigens or epitopes, to provoke an immune response in the body of affected animal/human. One of the most common immunogenic effects is the development of autoantibodies, mainly anti-nuclear antibodies and anti-double-stranded DNA antibodies, that have been associated to clinical manifestations of autoimmune diseases such as systemic lupus erythematosus with central nervous system vasculitis [74], scleroderma, autoimmune hepatitis and Sjogren syndrome [74,75]. Demyelination pathologies, such as multiple sclerosis and Guillain Barré syndrome have been associated also with TNF- α inhibitor therapies [76,77]. Etanercept therapy has been associated to the development of anti-etanercept antibodies; it seems to have essentially the same side effects of antibodies against TNF- α [78].

The Cochrane review entitled, 'Anakinra for RA' [79]. Evaluated the clinical effectiveness and safety of anakinra in adult patients with RA, using data from 2876 patients, from five trials which constituted in total 781 randomized to placebo and 2065 to anakinra. The authors concluded, "There were no statistically significant differences noted in most safety outcomes with treatment with anakinra versus placebo - including number of withdrawals, deaths, adverse events (total and serious), and infections (total and serious)". Injection site

reactions were significantly increased, occurring in 1235/1729 (71%) versus 204/729 (28%) of patients treated with anakinra versus placebo, respectively. These injection site reactions last for no more than four months and are not serious". Each of the trials reviewed measured prevalence of malignancies and found no evidence that these are increased amongst patients taking anakinra. These results should be taken into account when considering biologic therapy for patients with RA [80]. A modest increase in the risk of lymphoma and some solid tumors beyond background has been observed [81]. An increased incidence of serious infections and an increased risk of neutropenia have been observed when anakinra and etanercept were used concomitantly in patients with RA [82]. Similar interactions can be anticipated for the combination therapy of anakinra together with other agents blocking TNF- α (e.g., adalimumab, infliximab). Therefore, combined drug therapy with anakinra and any TNF-blocking agent is not recommended and should be avoided.

The methotrexate + anakinra co-administration has not provided evidence of increased toxicity [83]. In animal models studying the effects of both drugs when co-administered, no effects on clearance of both drugs from plasma or on the respective toxicological properties have been observed [84]. Therefore, the safety issue from concomitant use of both disease modifiers in patients with RA may be considered relatively secure.

Live-virus vaccines should not be administered to patients during treatment with anti-TNF- α BTs. Currently, information is not available whether or not these vaccines may affect the rate of secondary transmission of vaccine virus (e.g., measles or poliomyelitis viruses) following administration of a live-virus vaccine or regarding any other effect of vaccination on patients receiving the drug [85,86].

The application of tocilizumab is contraindicated during acute infections, as well as under latent tuberculosis. The most common ADEs observed in clinical trials were upper respiratory tract infections (more than 10% of patients), nasopharyngitis (common cold), headache, and high blood pressure (at least 5%). The enzyme, alanine transaminase (ALT), was also elevated in at least 5% of patients, but in most cases without symptoms. Elevations in ALT may be linked to viral hepatitis, diabetes, congestive heart failure, liver damage, infectious mononucleosis and myopathy. Among the less common side effects were dizziness, various infections, as well as reactions of the skin and mucosae like mild rashes, gastritis and mouth ulcer. Rare but severe reactions were gastrointestinal perforations (0.26% in six months) and anaphylaxis (0.2%) [59]. There are no certain indications of interactions with other drugs. The blood plasma levels of simvastatin, used for treatment of high levels of blood fats, were reduced by 57% after a single dose of tocilizumab, but it is not known whether this is clinically relevant observation. A possible mechanism is that the elevated IL-6 levels of patients with RA suppress the biosynthesis of various cytochrome P450 enzymes, notably CYP1A2, CYP2C9, CYP2C19 and CYP3A4. Tocilizumab lowers IL-6 and thus normalizes cytochrome levels, increasing the metabolism of simvastatin, and possibly other cytochrome metabolised drugs [59].

Pharmacovigilance and Drug Safety

Pharmacovigilance is associated with the observation, collection, detection, assessment, analysis, monitoring and prevention of ADEs and adverse drug reactions (ADRs). It is maintained by the programs established for the control and

vigilance of commercialized therapeutic agents. Pharmacovigilance was instigated in order to ensure drug safety and therapeutic efficacy within national and international populations following the introduction of new chemical entities (NCEs) in the laboratory. Clinical experimental phases are performed on restricted groups of patients and so when the biological therapeutic is distributed to general population, side and unwanted effects may be manifested. Thus, pharmacovigilance may be defined as a permanent monitoring system representing the post marketing experimentation. The expression “drug vigilance” (i.e. pharmacovigilance) was coined by two French pharmacologists in the second half of the 70's. Currently, the term, pharmacovigilance is accepted through all European and Global agencies (cf. European Commission for Public Health; World Health Organisation). The objective of drug vigilance is to monitor control the risk/benefit profiles of therapeutic compounds to ensure that the drug will always be to the benefit of the patients' health [87]. The concept of Adverse Event Reporting System (AERS) is central to pharmacovigilance whereby pharmacoepidemiology is the assessment of the incidence of adverse drug reactions in patient populations using drug agents. AERS involves a computerized information database designed to support the U.S. FDA post-marketing safety surveillance program for all approved drug and therapeutic biologic products. They are utilized by the FDA to monitor for new adverse events (AEs) and medical mishaps/negligence that might occur with the currently marketed products. It is necessary to signal and collect in a single database all eventual adverse reactions to drugs (ADRs) observed on national territory to realize a correct evaluation of the security and efficacy profile.

The Italian system for pharmacovigilance, is based on the insertion of ADRs in the national network of pharmacovigilance (RNF) directly controlled by AIFA (Agenzia Italiana del Farmaco; Drug Italian Agency). Periodically, all national data are sent to the European Commission database (Eudravigilance) controlled by the drug European Agency. The analysis of ADR signaling inserted in the different drug vigilance databases, facilitates the identification of drugs dangerous for the patient health or information concerning specific categories of patients such as the elderly, children or pregnant women [88]. In the case of risk/benefit variations the agencies proceed to a new evaluation of the products that may lead to a modification of the drug sheet or, if necessary, to the withdrawal of the drug from commerce.

Another important objective of pharmacovigilance is monitoring eventual drug interactions difficult to be evaluated if only based on randomized clinical trials obliged for the achievement of the authorization to marketing (AIC) [89]. Initiatives capable of guaranteeing active drug vigilance and communication among AIFA and the different Italian Regional administrations are promoted for this purpose. The pharmacovigilance administrations among regional agencies periodically meet at AIFA congresses to coordinate operative ways associated with fixed tasks. Furthermore, the Annual Report on Pharmacovigilance is presented to the Italian Government by Istituto Superiore di Sanità (ISS) and AIFA.

The Italian Ministry of Labor, Health and Social Politics in collaboration with Regional Administrations and the AIFA develop guidelines according to those originating from the European Community that are designed specifically for professionals in the Public Health Domain. Physicians, medical informants, pharmaceutical

companies, pharmacists, citizens, nurses, health workers, may all signal adverse drug events. A signaling schedule may be inserted with hospitals, ASL (Aziende Sanitarie Locali; Local Health Agencies), pharmaceutical marketing, or downloaded from the AIFA web site. Reports are then sent to the industry that commercializes the drugs.

Pharmacovigilance among Biological Therapeutics Affecting Immune Response

Pharmacovigilance is particularly important for the analytic consideration all BTs. These compounds are administered by the clinicians who also prescribe traditional therapeutic agents, thus comparisons between both types of interventional agents may be obtained routinely.

In the last decade, much attention has been focused upon BTs with the advent of novel NCEs and their commercial applications. Like all pharmaceutical agents, BTs undergo traditional pre-approval analyses. Initially, the security profile of biological drugs is not completely understood and the analysis of its distribution and utilization among patients facilitates the identification adverse side effect profiles. For example, a wider drug distribution monitoring may provide information leading to an alteration of recommended dosages, provided that the compound has passed drug safety analysis.

A multitude of factors may influence the immune response against the administered drug therapies: the protein structure, its glycosylation, the type of formulation and the catabolic products it can generate and at the same time the dosage, the treatment schedule, the way of administration and the use of other immunomodulators, etc. In some cases, the development of an immune response to

BTs may reduce the efficacy of the molecules accompanied by only mild adverse effects. Since BTs reduce the immune response to antigens in general, vaccine efficacy may be reduced in patients [86]. In other cases, the immune response may lead to serious and lethal outcomes. For example, in hematology, anti-erythropoietin antibodies have in some cases caused the development of a severe aplasia, retarded or defective development, of the red blood cells [90].

Other complications arising from treatment with biological therapeutic agents are immunocomplex diseases, such as glomerulonephritis, vasculitis and arthritis. Consequently, when ADEs are analysed in patients treated with BTs immunogenicity and immune-mediated reactions must be taken into consideration. Anaphylactic immunoglobulin E (IgE) linked reactions and anaphylotoid non-IgE linked reactions may appear as a consequence of the infusion of BTs. It is important to distinguish this event from the reaction to the infusion.

Immunosuppressor and immunomodulator therapeutic agents bear the risk for opportunistic infections and tumors [91]. Pharmacovigilance systems are able to signal ADEs that appear shortly after the administration of the therapeutic agent, but they are inadequate to reveal ADEs that appear months or years following treatment. It is important to analyze comprehensively those situations wherein the agenda is reviewed for opportunistic infections and tumors. For this reason, journals monitoring patients treated with immunosuppressors and immunomodulators have been introduced. This Journal register presents a review over all occasions when an opportunistic process has occurred in monotherapy, or when a product has been administered in association to other immunosuppressors and immunomodulators. These

ancillary Journal registers provide information regarding the frequency and development of ADEs.

As yet, it is not certain whether or not ADEs occur more often in patients treated with BTs compared to traditional medication drugs; nevertheless, the analysis of results from the USA and Europe, and in particular from Biomedical Authorities in Italy, suggests that the probability of a “regulatory action” for a BT was 14%, three years after the distribution of the therapeutic agent in the population, and was 29%, ten years later. In most cases, the regulatory actions were associated with local reactions at the infusion site, infections, tumors and immune system disturbances. Following ten years of administration, 17% of the BTs have been awarded a “boxed warning” for insertion into the technical schedule [92,93].

Conclusion

The introduction of BTs offers several types of intervention for autoimmune disease states: cytokine-blockers, anti-TNF- α agents, monoclonal antibodies, soluble receptors, antagonists of IL-1 and IL-6 and agents that reduce circulating B-lymphocytes and counteract T-lymphocyte activation. For example, in systemic lupus erythematosus, targeted immunotherapies, such as the anti-B cell activating factor (anti-BAFF) antibody, belimumab, has been approved as an add-on therapy for patients who have active disease despite receiving standard therapy [94]. The principle of pharmacovigilance postulates that BTs require ‘vigilant’ monitoring both for approval and incidence of adverse immune-mediated events, reaction infusion and occurrence of opportunistic infections and tumors.

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