Review

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Biological therapies in rheumatic diseases

F. Conti, F. Ceccarelli, L. Massaro, E. Cipriano, M. Di Franco, C. Alessandri, F.R. Spinelli, R. Scrivo, G. Valesini

Department of Internal Medicine and Medical Specialties, Rheumatology, 'Sapienza' University, Rome, Italy

Abstract

The development of the biological drugs has revolutionized the therapeutic approach of the chronic inflammatory rheumatic diseases, particularly in patients resistant to standard treatment. These drugs are characterized by an innovative mechanism of action, based on the targeted inhibition of specific molecular or cellular targets directly involved in the pathogenesis of the diseases: pro-inflammatory cytokines (tumor necrosis factor, interleukin-1 and 6), CTLA-4, and molecules involved in the activation, differentiation and maturation of B cells. Their use has indeed allowed for a better prognosis in several rheumatic diseases (such as rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, systemic lupus erythematosus) and to obtain a clinical remission. In the present review we give an overview of the biological drugs currently available for the treatment of the rheumatic diseases, analyzing the different mechanism of action, the therapeutic indications and efficacy data, and adverse events. ***Clin Ter 2013; 164(5):e413-428.*** **doi: 10.7417/CT.2013.1622**

Key words: *ankylosing spondylitis, biological drugs, psoriatic arthritis, rheumatoid arthritis, systemic lupus erythematosus*

Introduction

In the past decades several studies have highlighted the role of pro-inflammatory cytokines in the pathogenesis of chronic inflammatory rheumatic diseases. As a result, a more concentrated effort has been made to develop drugs targeting the molecules directly involved in the inflammatory response (Table 1).

The development of these drugs, called biologics, has revolutionized the therapeutic approach of the chronic inflammatory rheumatic diseases, particularly in patients resistant to standard treatment. Their use has indeed allowed for a better prognosis, also leading to clinical remission in some patients.

The history of biological drugs began in 1975, when Köhler and Milstein developed the method for isolating monoclonal antibodies (mAbs) from hybridoma cells (1). The first step in the production of antibodies against specific molecules was the cloning of murine genes of variable heavy (VH) and variable light (VL) chains. It was then possible to synthetize chimeric antibodies, containing the murine VH and VL chains fused with the constant region of human origin (2). More specifically, antibodies obtained by this technology show approximately one-third murine and two-thirds human sequences. However, the efficacy of murine-derived immunoglobulin preparations could be limited by the induction of anti-mouse immune responses, with consequent impairment of the therapeutic efficacy. Hence, the antibodies of recent development are as human as possible. Another problem, which still remains unsolved, despite the numerous studies performed, pertains to pharmacodynamic and pharmacokinetic aspects of mAbs. This is probably due to their inter-individual variability and extension of the inflammatory mechanisms underlying the disease process. Generally, the half-life of these drugs increases with the degree of humanization, but many different mechanisms, such as the proteolytic degradation and the glycosylation, may also play an important role in their clearance (3).

To overcome these limitations, other biological constructs have been tuned, such as recombinant molecules or polyethylene glycol (PEG)-fused molecules. They do not require non-human amino acid sequences, which minimize the antigenic potential while PEGylation avoids potential Fc-mediated effects, enhances solubility and half-life *in vivo*, and may contribute to its preferential distribution to inflamed tissues (4-6).

Major advances in biotechnology alone do not explain the growing availability of biological agents; the improved understanding of the pathogenesis of chronic inflammatory diseases has also played an important role, leading to identify several targets: the pro-inflammatory cytokines tumor necrosis factor (TNF), interleukin-1 (IL-1), and IL-6, CTLA-4 (which modulates T cell activation), and molecules involved in the activation, differentiation and maturation of B cells. The biological agents targeting the aforementioned molecules are now available for many rheumatic diseases, including rheumatoid arthritis (RA), psoriatic arthritis (PsA),

Correspondence: Fabrizio Conti. Dipartimento di Medicina Interna e Specialità Mediche, Sezione di Reumatologia, Sapienza Università di Roma, Roma, Italy. Viale del Policlinico 155, 00161-Roma, Italia. Tel.: +39.06.49974631; Fax: +39.06.49974670.
E-mail: fabrizio.conti@uniroma1.it

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Table 1. Main characteristics of the anti-TNF agents currently available.

Drug	Structure	Target	Approved Indications	Route, Dose and Frequency of Administration
Infliximab (Remicade®)	Mouse/human chimeric IgG1 mAb	Soluble and membrane TNF	RA/AS/PsA	Intravenous infusion, 3 to 5 mg/kg at 0, 2, 6 weeks, then every 6-8 weeks
Etanercept (Enbrel®)	Human sTNFR2-Fc fusion protein	Soluble and membrane TNF	RA/AS/PsA	Subcutaneous injection, 25 mg twice a week or 50 mg once a week;
Adalimumab (Humira®)	Human IgG1 mAb	Soluble and membrane TNF	RA/AS/PsA	Subcutaneous injection, 40 mg every 2 weeks
Golimumab (Simponi®)	Human IgG1 mAb	Soluble and membrane TNF	RA/AS/PsA	Subcutaneous injection, 50 mg every 4 weeks
Certolizumab pegol (Cimzia®)	PEG-human IgG1 mAb fragment (Fab)	Soluble and membrane TNF	RA	Subcutaneous injection, 400 mg at 0, 2, 4 weeks, then 200 mg every 2 weeks

Legend: TNF: tumor necrosis factor; mAb: monoclonal antibody; PEG: polyethylene glycol; RA: rheumatoid arthritis; AS: ankylosing spondylitis; PsA: psoriatic arthritis.

ankylosing spondylitis (AS), systemic lupus erythematosus (SLE), autoinflammatory diseases. In order to optimize their use in the clinical practice and because of their significant cost, the main rheumatological scientific societies have published and periodically updated specific guidelines/recommendations. However, there are some diseases where trials have not been able to demonstrate a significant improvement by using the current treatment: in these cases, only the clinical evidence and the experience of the phisician may drive the therapeutic decision.

We now review the main biological drugs making a classification based on the targeted mechanism of action.

TNF antagonists

TNF is a cytokine implicated in many aspects of the inflammatory processes. It is released from several different immune and non-immune cells as a soluble molecule after being enzymatically cleaved from the cell surface. Both soluble (sTNF) and membrane TNF (mTNF) are biologically active when interact with either of two distinct receptors, TNF receptor 1 (TNFR1, p55) and TNFR2 (p75), expressed on a wide variety of cells (7). A lot of studies have demonstrated the key role of TNF in the pathogenesis of chronic inflammatory diseases such as RA, PsA, AS, inflammatory bowel diseases, and uveitis (8).

As a consequence, starting from the late 90's five different drugs targeting TNF have been developed, which dramatically ameliorated the outcome of the patients: infliximab, adalimumab, golimumab, certolizumab pegol, which are mAbs or fragments thereof, and etanercept, a genetically engineered fusion protein composed of a dimer of the extracellular portions of human TNFR2 fused to the Fc portion of a human IgG1. Infliximab, adalimumab and golimumab are full-length, bivalent IgG1 mAbs, whereas certolizumab is a monovalent Fab1 antibody fragment covalently linked to PEG. Infliximab is a chimeric protein containing ~25% mouse-derived amino acids comprising the VH and VL domains; certolizumab is a humanized protein containing amino acid sequences derived from a mouse anti-TNF mAb and inserted into human VH and VL domains; adalimumab and golimumab are fully human mAbs. Infliximab, adalimumab and golimumab are IgG1 antibodies, which are capable of complement fixation and Fc-receptor binding. Certolizumab is a Fab1 fragment of an IgG1 mAb and lacks effector functions because it has no Fc region (9).

Here we report the main characteristics of these compounds, focusing on their clinical profile.

Mechanisms of action

The mechanism of action of TNF antagonists is based on the neutralization of both sTNF and mTNF. The interruption of the signal pathways mediated by TNF has numerous consequences, reflecting the pleiotropic effect of the cytokine: cell cycle arrest, apoptosis, inhibition of pro-inflammatory cytokine and chemokine release, but also of chondrocyte, osteoclast, and endothelial cell activation, reduction of leukocyte accumulation and angiogenesis, increase of T reg cell number (10-20). TNF seems to be involved also in the modifications of lipid profile, since the treatment with anti-TNF agents produce increase of the HDL levels as well as of the total cholesterol, which are associated with a significant improvement in RA activity (21, 22).

Approved indications

Rheumatoid arthritis: RA was the first indication for the use of TNF antagonists. The main goals in the treatment of patients affected by RA are the control of the signs and symptoms, the prevention of joint damage progression and, the remission achievement (23). A large number of randomized controlled trials (RCT) has demonstrated the efficacy of all TNF antagonists in the treatment of RA (Table 2) (24-33).

In patients with RA, anti-TNF drugs have been used either in monotherapy or associated with methotrexate (MTX), internationally accepted as the first disease-modifying antirheumatic drug (DMARD) choice in the management of RA. TNF antagonists were generally tested versus MTX: some studies evaluated their efficacy in patients with early RA and naïve to MTX therapy, while other studies enrolled patients with established disease, not adequately responding to MTX. From these studies a conviction emerged, that when MTX was added to biologics the response rates were much higher than for the biologics by themselves. Several rheumatological scientific societies in their guidelines/

Table 2. Main randomized controlled trials testing the efficacy of anti-TNF agents in RA patients.

Study	Treatment	Treatment Duration	Outcome
COMET, Emery (24)	ETA + MTX vs MTX	52 weeks	Remission (DAS28), radiographic progression (van der Heijde-modified Sharp score)
TEMPO, Klareskog (25)	ETA + MTX vs ETA or MTX	52 weeks	ACR response, radiographic progression (van der Heijde-modified Sharp score)
ERA, Genovese (26)	ETA vs MTX	12 months	ACR response, radiographic progression (van der Heijde-modified Sharp score)
ATTRACT, Maini (27)	IFX + MTX vs MTX	30 weeks	ACR response
ASPIRE, St. Claire (28)	IFX + MTX vs MTX	54 weeks	ACR response, radiographic progression (van der Heijde-modified Sharp score)
ARMADA, Weinblatt (29)	ADA + MTX vs MTX	24 weeks	ACR response
PREMIER, Breedveld (30)	ADA + MTX vs ADA or MTX	2 years	ACR response, radiographic progression (modified Sharp score)
GO-AFTER, Smolen (31)	GLM 50 mg ± DMARDs vs GLM 100 mg or 100 mg ± DMARDs vs DMARDs	24 weeks	ACR response HAQ-DI, DAS28 (also remission), FACIT-F
GO-FORWARD, Keystone (32)	GLM 100mg + vs MTX + placebo vs GLM 50 mg + MTX vs GLM 100 mg + MTX	52 weeks	ACR response, DAS28, safety
RAPID-1, Keystone (33)	CZP 400 + MTX vs CZP 200 + MTX vs MTX	52 weeks	ACR response; radiographic progression (van der Heijde-modified Sharp score)

Legend: TNF: tumor necrosis factor; RA: rheumatoid arthritis; ETA: etanercept; MTX: methotrexate; ACR: American College of Rheumatology; IFX: infliximab; ADA: adalimumab; GLM: golimumab; DMARDs: disease modifying anti-rheumatic drugs; HAQ-DI: Health Assessment Questionnaire – Disability Index; DAS 28: disease activity score 28; FACIT-F: Functional Assessment of Chronic Illness Therapy - Fatigue; CZP: certolizumab pegol

recommendations identify RA patients deserving treatment with anti-TNF drugs also based on disease activity as assessed by validated measures, such as the disease activity score 28 (DAS28) (34). According to DAS28, the level of disease activity can be interpreted as remission (DAS28≤2.6), low (2.6<DAS28≤3.2), moderate (3.2<DAS28≤5.1), or high (DAS28>5.1) (34). For example, a committee of experts on behalf of the Italian Society for Rheumatology (Società Italiana di Reumatologia, SIR) recommend to use anti-TNF agents in RA patients with insufficient response to MTX**,** taken for at least 3 months in the highest tolerated dosage (up to 20 mg/week). In patients with contraindications or intolerance to MTX, the failure of another drug with structural efficacy must be proven. The failure of DMARDs is defined by a high disease activity (DAS28>5.1) or even a moderate disease activity (3.2<DAS28≤5.1) in the presence of unfavorable prognostic factors or after failing a combination or sequential administration of various DMARDs (35). According to SIR recommendations, anti-TNF agents may also be initiated in patients with evidence of joint damage progression regardless of disease activity (35). Patients not achieving EULAR response (using DAS28) after 12 weeks of biological treatment should be considered non-responders and a change in the treatment strategy is recommended (35).

Despite the efficacy of TNF antagonists, approximately one third of patients discontinue the treatment due to inefficacy or intolerance (36). In these cases, the switching to another anti-TNF agent could represent a valid option because of significant differences in terms of molecular structure, pharmacokinetics, interactions with TNF, generation of antibodies, induction of apoptosis, and dosing regimen among the TNF antagonists (37, 38). The analysis of studies evaluating the efficacy of switching strategy demonstrates that a good disease control may be obtained with a second anti-TNF agent, especially in patients withdrawing the first drug for loss of response during time or adverse events. Conversely, patients stopping the first TNF antagonist because of lack of efficacy are more likely to respond to biologics recognizing targets other than TNF (39). Finally, no univocal data are available concerning the duration of anti-TNF treatment, but it has been observed that the discontinuation of the therapy, often during a long-lasting remission period, is almost always followed, after a variable period, by disease reactivation (40).

Spondyloarthritis: PsA and AS are the two entities with the most severe course of all SpAs, and several RCT testing TNF antagonists have been run in such patients demonstrating an impressive clinical efficacy, with no specific superiority in terms of efficacy of one of them over the others (Table 3) (41-49).

Nowadays, four TNF antagonists (infliximab, etanercept, adalimumab, and golimumab) are licensed for treatment in patients with PsA and AS in case of a non-response to other therapies. As for RA, aims of therapy in SpA are the reduction of inflammation, inhibition of radiologic progression, preservation of joint function, and improvement of quality of life. Recommendations for the use of biological agents in

PsA and AS have been suggested by several rheumatological scientific societies, including SIR (50). For clinical purposes, PsA is generally classified into two main types: one with a predominant peripheral joint involvement and the other with predominant axial manifestations, and these are associated with different therapeutic strategies. According to SIR recommendations, anti-TNF therapy should be considered in patients with active PsA predominantly characterized by peripheral synovitis that failed conventional treatment (non-steroidal anti-inflammatory drugs -NSAIDs- and at least 1 DMARD administered alone or in combination for at least 3 months). As recommended for RA, PsA patients may also be considered for anti-TNF therapy in case of progression of joint damage documented by conventional X-rays, even though they have an acceptable clinical response. For patients with psoriatic spondylitis, instead, the failure of at least 2 NSAIDs taken over a 3-months period to maximal doses is sufficient to initiate treatment with anti-TNFs. For both subtypes of PsA, response to biological therapy should be assessed 3 months after treatment onset based on expert opinion, evaluation of clinical symptoms and signs, of acute phase reactants, and of imaging studies whenever appropriate (50). All available anti-TNFs can be used in monotherapy with similar clinical efficacy, but in case of failure, the switch to another anti-TNF may be an option (50, 51). The considerations related to the axial form of PsA are very similar to those established for the treatment of AS by an expert group (52). NSAIDs are the first-line treatment in patients affected by AS, because DMARDs have not been shown to be effective in the control of axial manifestations. Several studies demonstrated the efficacy of anti-TNF drugs in reducing inflammation status and improving the quality of life of AS patients with no specific superiority in terms of efficacy of one of them over the others (Table 3). Interestingly, all available anti-TNFs are effective in inducing a significant clinical improvement in a short time (about 2 weeks) (53, 54). In patients with concomitant inflammatory bowel disease and/or uveitis, the monoclonal antibodies have shown to be more effective than the fusion protein (55, 56). An analysis of over 800 AS patients from the Danish registry documented a rapid and sustained decrease in disease activity after treatment with TNF antagonists, especially in men, with only few patients stopping treatment owing to adverse effects (57). Nearly one-third of AS patients in clinical practice switch biological treatment and the new anti-TNF, as in RA and PsA patients, may prove successful (43-46, 58).

Peculiar Use of TNF antagonists

Considering the evidences of high expression of TNF at synovial membrane level, the use of intra-articular injections proved to give encouraging results in patients with RA or SpA with refractory monoarthritis. The synovitis, evalua-

Table 3. Main randomized controlled trials testing the efficacy of anti-TNF agents in AS and PsA patients.

Study	**Disease**	**Treatment**	**Treatment duration**	**Outcome**
Braun (41)	AS	IFX vs placebo	12 weeks	BASDAI, BASFI, BASMI, SF-36
ASSERT, van der Heijde (42)	AS	IFX vs placebo	24 weeks	ASAS response, BASDAI, BASFI, BASMI, SF-36, CRP, SJC, MEI
Davis (43)	AS	ETA vs placebo	24 weeks	ASAS response, safety
van der Heijde (44)	AS	ADA vs placebo	24 weeks	ASAS response, BASDAI, BASFI, BASMI, CRP, SJC, TJC
GO-RAISE, Inman (45)	AS	GLM 50 mg vs GLM 100 mg vs placebo	24 weeks	ASAS response, BASDAI, BASFI, BASMI, SF-36
IMPACT, Antoni (46)	PsA	IFX vs placebo	50 weeks	ACR response, PASI, DAS28, HAQ, PsARC
Mease (47)	PsA	ETA vs placebo	12 weeks	ACR response, PsARC, PASI
Mease (48)	PsA	ADA vs placebo	24 weeks	ACR response, radiographic progression (modified Sharp score), PsARC, PASI, HAQ, SF-36
GO-REVEAL, Kavanaugh (49)	PsA	GLM 50 mg vs GLM 100 mg vs placebo	24 weeks	ACR response, PASI, SF-36, HAQ, NAPSI, MASES

Legend: TNF: tumor necrosis factor; AS: ankylosing spondylitis; IFX: infliximab; ASAS: Assessment in Ankylosing Spondylitis; CRP: C-reactive protein; SJC: swollen joint count; MEI: Mander Enthesis Index; ETA: etanercept; ADA: adalimumab; TJC: tender joint count; GLM: golimumab; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; BASMI: Bath Ankylosing Spondylitis Metrology Index; SF-36: Short-Form 36; PsA: psoriatic arthritis; ACR: American College of Rheumatology; PASI: Psoriasis Area and Severity Index; PsARC: Psoriatic Arthritis Response Criteria; HAQ: Health Assessment Questionnaire; NAPSI: Nail Psoriasis Severity Index; MASES: Maastricht Ankylosing Spondylitis Enthesitis Score

ted by using ultrasonography and scintigraphy, showed a significant improvement after long-lasting follow-up (59-62). Moreover, TNF-antagonists could be useful in other inflammatory rheumatic diseases resistant to conventional therapies, such as Behcet's disease (63, 64).

Adverse events

TNF plays a crucial role in the defense against microbial agents. Therefore, when its effects are blocked, patients may be at higher risk of infections and indeed an increased risk of developing infections in the upper and lower airways and urinary tract has been registered (65). Most importantly, TNF inhibition may favour the reactivation of latent tuberculosis infection (LTBI) in previously exposed patients, and this is the reason why an appropriate screening should be carried out in all patients undergoing treatment with anti-TNFs (66). This consists of tuberculin skin test (TST), chest radiography, medical history focused on risk factors for TB, and physical examination. Since TST lacks sensitivity and specificity, especially in immunocompromised people, novel screening tools, the IFN-γ release assays (IGRAs), have been introduced (67, 68). These tests are more specific than TST, but an optimal screening strategy at the moment should include both TST and an IGRA to maximise the possibility of identifying patients already infected by *Mycobacterium tuberculosis* (69). Patients with a positive screening for LTBI must be treated with antitubercular drugs for 9 months starting one month before biological treatment (70). Another major problem concerns HBV-positive patients, which may experience raised liver function tests, increase of viral load and fatal hepatic failure under anti-TNF treatment, therefore HBV screening tests must be perfomed before starting biological treatment (71). On the contrary, in HCV-infected RA patients, several short-term observational studies have shown no clear worsening or reactivation of viral disease associated with anti-TNF therapy, and the prophylactic use of antiretroviral agents is not mandatory (72). Apart from the risk of infections, another source of concern was related to the possible occurrence of malignancies, since patients with autoimmune diseases have an increased risk of developing lymphomas when compared with the healthy population (73). Data analyzed so far do not indicate an increased risk of developing lymphomas in patients exposed to TNF inhibitors, but the clinical trials examined were not adequately powered to address this issue and few reports have been published (74, 75). Likewise, the occurrence of solid malignancies with anti-TNF is not increased in RCTs and in long-term observational studies, with the exception of an increased risk of non melanoma skin cancers (74). A decreased risk of cardiovascular events in patients treated with TNF blockers was observed (76), but in case of advanced chronic heart failure (NYHA classes III and IV) their use is contraindicated since it was associated with increased morbidity and mortality (77, 78).

The role of anti-TNF remains obscure in regards to the appearance of neurological disorders. The most commonly identified related alterations are central and peripheral demyelinating lesions, but short-term follow-up indicates relatively good outcomes, sometimes after biologic discontinuation or after glucocorticoids or intravenous immunoglobulin treatment (79). A paradoxical adverse event secondary to the use of anti-TNFs is the exacerbation of preexisting psoriatic lesions and new-onset psoriasis: in the majority of cases the local treatment of psoriatic lesions allowed to continue anti-TNF therapy, although in more severe cases switching to another anti-TNF agent or withdrawal of the biologic treatment is necessary (80, 81).

The use of anti-TNF agents has been also associated with laboratory abnormalities: haematological dyscrasias such as aplastic anaemia, pancytopenia and neutropenia have been very rarely described, while it is more frequent the occurrence of non-organ specific auto-antibodies such as antinuclear (ANA), anti-phospholipid (aPL) and anti-double-stranded DNA antibodies (anti-dsDNA) (82). However, related clinical autoimmune syndromes are rare and mostly reversible after anti-TNF treatment withdrawal. Furthermore, anti-TNFs, including full human ones, are by themselves immunogenic, leading to the induction of anti-drug antibodies that can be associated with therapeutic failure and side effects (83).

Anti-IL-1 agents

The superfamily of IL-1, constituted by pro-inflammatory cytokines, receptors and antagonist molecules, is involved in the regulation of the innate immunity. Several evidences demonstrated a modification of the balance of these molecules during the course of many autoinflammatory and autoimmune diseases. IL-1α and IL-1β, synthesized by mononuclear cells, are the major cytokines of the group. Two receptors mediate the action of IL-1, IL-1 receptor type I (IL-1RI) and IL-1RII, and also an antagonist of these receptors has been identified (IL-1Ra). To date, different strategies to block the action of IL-1 have been developed. Anakinra is a recombinant non-glicosylated form of the IL-1Ra (Table 1). This drug was approved in 2001 for the treatment of patients affected by RA and later for other diseases. It is administrated subcutaneously at a dose of 100 mg daily, but an intravenous administration could be performed, especially in case of acute onset of the disease. The half-life of anakinra is short, about 6 hours, requiring daily administration (84). More recently, other drugs targeting IL-1 were developed and tested. Canakinumab, a mAb against IL-1β currently investigated in phase III studies, has been approved for the treatment of systemic onset juvenile idiopathic arthritis and cryopyrin-associated periodic syndrome (85). Rilonacept (also known as IL-1 Trap) is a recombinant fusion protein consisting of the extra-cellular ligand-binding domains of human IL-1RI and IL-1R accessory protein, fused to the Fc portion of human IgG1. It acts as a soluble decoy receptor, trapping both IL-1α and IL-1β with high affinity. Rilonacept was approved for the treatment of CAPS, familial cold auto-inflammatory syndrome, and Muckle-Wells syndrome (86).

Mechanism of action

IL-1α mainly acts in an autocrine fashion and partially by exerting a paracrine function, which result in local inflammation. Conversely, IL-1β is released into the circulation and stimulates systemic inflammation. The two receptors

mediating their action, IL-1RI and IL-1RII, are expressed on the macrophages and B lymphocyte surfaces as a membrane receptor and also released in a soluble form. The binding of IL-1 to its receptor initiates the recruitment of several kinases with development of the pro-inflammatory cascade (87). The main functions of IL-1 are the activation of immune cells, particularly neutrophils, the stimulation of the secretion of colony stimulating factors, and the promotion of the differentiation of T helper (Th) lymphocytes in Th17. In addition, IL-1 activates endothelial cells, synovial fibroblasts, and osteoclasts, and stimulates the chondrocytes to produce matrix degrading enzyme. Finally, IL-1 acts on the endocrine system, especially on the hypothalamic-pituitary axis, promoting the release of ACTH, GH, ADH, somatostatin, and affects glucose metabolism (84, 87). The IL1-Ra is a glycosilated protein of 22kD that antagonizes the activation of the IL-1R. Its expression is inducible in many cells, while it is constitutively expressed in keratinocytes and intestinal epithelial cells. IL-1Ra binds with high affinity to IL-1R, preventing the transmission of signals (88). Experimental data demonstrated that IL-1Ra knockout mice develop an inflammatory erosive arthritis with clinical and histological features similar to those in RA. In addition, in these mice the levels of Th17 were increased (86).

Approved indications

Rheumatoid arthritis: Anakinra, alone or in combination with MTX, resulted effective in the reduction of disease activity and damage and in the improvement of the quality of life (89). After 16 weeks of treatment with anakinra, a significant improvement in signs, symptoms and laboratory parameters, as well as a slowing of radiographic progression, was registered in RA patients (89). Despite the absence of clinical trials directly comparing anakinra with respect to TNF antagonists, the experience clearly demonstrates a superiority of the TNF blocking strategy in RA.

Adverse events

Anakinra is characterized by a good safety profile: the reactions at the injection site are the most common adverse effects, probably related to daily administration. Furthermore, the use of anakinra results in an increase in bacterial and viral infections, especially of the upper airways, and in a reduction of circulating neutrophils, even if rarely in a severe neutropenia (less than 500 mm^3) has been described. In these cases the number of neutrophils increased shortly after discontinuation of the drug (84, 90). No data are available regarding the development of malignancies during the treatment (84, 90).

Anti-IL-6 agent

IL-6 is a pleiotropic cytokine that plays a key role in the inflammatory processes by inducing the activation of several cells involved in immune response. It acts by means of interaction with its receptor (IL-6R), composed of two chains. The first chain, formed by a domain containing the binding site for IL-6, could exist in soluble form or associated with the second chain. This is a glycoprotein of 130 kD, located on the membrane of different cell types (91). The binding of the glycoprotein complex IL-6/IL-6R leads to enrollment of JAK kinases, with the activation of transcription factors, such as STAT3 and SHP2, and the modulation of the gene expression in pro-inflammatory sense. Tocilizumab is a humanized mAb of IgG1 class against IL-6R, that prevents the formation of the IL-6/IL-6R complex. Tocilizumab is administrated intravenously at a dose of 4 or 8 mg/kg every 4 weeks. The half-life of the drug is concentration-dependent: about 11 days in case of 4 mg dosage, 13 days in case of 8 mg dosage. Tocilizumab can be used in monotherapy or in combination with MTX and it is metabolized by the reticulo-endothelial system as an endogenous immunoglobulin (92).

Mechanism of action

IL-6, produced by monocytes and macrophages as a consequence of Toll-like receptors (TLRs) stimulation, acts directly on immune cells by promoting the differentiation of B cells, the proliferation of T cells (especially the differentiation of T CD4+ in Th17 and T CD8+ in cytotoxic cells), the suppression of T reg, and the activation of macrophages. Furthermore, IL-6 acts on the hepatocytes with an increase of the acute phase proteins production leading to the recruitment of leukocytes in the joints, proliferation of synoviocytes and release of metalloproteinases (93). The IL-6 effects on osteoblasts, endothelial and mesangial cells, fibroblasts and keratinocytes determine the cartilage and subchondral bone degradation and loss of systemic bone. Moreover, an increase of the collagen synthesis was reported, contributing to skin changes that occur in psoriasis and systemic sclerosis (91, 94). High concentrations of IL-6 have been demonstrated in serum and synovial fluid of patients affected by RA. In the synovial fluid the IL-6/IL-6R complex induces the formation of osteoma-like cells and in bone marrow induces the activation of the RANK/RANKL complex. Moreover, IL-6 increases the production of VEGF that results in an increase of angiogenesis and of the synovial permeability.

Approved indications

The use of tocilizumab was approved in RA patients with moderate/severe disease activity, both as first-line therapy after failure of DMARDs, or after the failure of TNF inhibitors (93). In Table 4 the main clinical trials in which tocilizumab was used in RA patients are reported (92-95). The response to treatment with tocilizumab is comparable to that of other biologics in terms of ACR response (92-95). Particularly, in the SAMURAI study, tocilizumab has proven effective in reducing joint damage (92).

Adverse events

Tocilizumab is characterized by a good safety profile. Infections are the most common AEs, although serious outcomes are rare. Upper respiratory tract infections and pharyngitis are the most commonly reported, while the serious events are represented by pneumoniae, urinary tract infections, cellulites, herpes zoster. Cases of TB have been observed, so patients should be screened for latent

Table 4. Main randomized controlled trials testing the efficacy of tocilizumab in RA patients.

Study	Treatment	Treatment duration	Outcomes
SAMURAI, Nishimoto (92)	TCZ vs DMARDs	52 weeks	Radiographic progression, (modified Sharp score), ACR response, DAS, HAQ
AMBITION, Jones (95)	TCZ vs MTX	24 weeks	ACR response, HAQ, DAS28
OPTION, Smolen (93)	TCZ 4 mg + MTX vs TCZ 8 mg plus MTX vs MTX	24 weeks	ACR response, DAS28, HAQ, SF-36, FACIT-F
SATORI, Nishimoto (96)	TCZ vs MTX	24 weeks	ACR response, DAS28, HAQ, VEGF levels
CHARISMA, Maini (97)	TCZ 2 mg/4 mg/8 mg ± MTX vs MTX	20 weeks	ACR response, DAS28, CRP/ESR levels

Legend: RA: rheumatoid arthritis; TCZ: tocilizumab; DMARDs: disease modifying anti-rheumatic drugs; ACR: American College of Rheumatology; DAS: disease activity score; HAQ: Health Assessment Questionnaire; MTX: methotrexate; SF-36: Short Form 36; FACIT-F: Functional Assessment of Chronic Illness Therapy-Fatigue; VEGF: vascular endothelial growth factor; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

TB before treatment (92, 93, 95). A decrease in neutrophil counts (<1.000/mm^3) can also occur. In the majority of cases, neutropenia is transient, without the need of drug discontinuation. Moreover, no clear relationship between decreases in neutrophils and occurrence of serious infections was found (92, 93, 95-98).

A modification in lipid profile, as increase in the concentration of total cholesterol, LDL, HDL and triglycerides, was observed during treatment. Hence, the lipid profile should be evaluated after 1-2 months of therapy starting and then every 6 months. However, these changes seem to respond well to statins. No data are available regarding long-term effects on cardiovascular function. Moreover, tocilizumab determine an increased risk for elevating liver enzyme levels. Infusion-related events are generally mild and transient. Among these, hypertension, headache and skin reactions are the most commonly reported within the first 24 hours of infusion (92, 93, 95-98). Bowel perforation, followed by peritonitis has been reported. Due to the increase risk of perforation, tocilizumab must be administered with caution in patients with a history of ulcer or diverticulitis (99). Moreover, IL-6 may also assist wound healing indirectly by modulation of growth factors or their receptors. This evidence could explain the delay of wound healing in patients treated by tocilizumab (100).

Co-stimulation signal blockade

The activation of naïve T lymphocytes and the differentiation into effector T cells require at least 2 signals. The first one is mediated by the TCR, while the second is a co-stimulatory signal necessary for the full activation. The most important co-stimulatory molecules are a pair of related proteins, CD80 and CD86, expressed by dendritic cells, macrophages, and B cells. The complex CD80/86 is recognized by specific receptors localized on the surface of T lymphocytes. The first receptor is CD28 and its activation drives the signals leading to the expression of pro-inflammatory genes (101). The CTLA-4 is the second receptor that binds CD80/86. It is structurally homologous to CD28 but its main function is to inhibit the activation of T cells counteracting signals from the TCR and CD28. CTLA-4 is constitutively expressed in CD4+CD25+ cells and is inducible in activated T cells. CTLA-4 binds CD80/86 with a higher affinity compared with CD28, with consequent inhibition of the immune response, and in particular the inhibition of IL-2 production and progression of the cell cycle. Secondly, CTLA-4 promotes the suppressive action of T reg and is involved in the maintenance of T tolerance (101-103). Abatacept, a drug able to block T cell co-stimulation, is a dimeric fusion protein consisting of the extracellular domain of CTLA-4 fused with the modified Fc portion of a human IgG1. Abatacept is administered intravenously at a dose of 10 mg/kg at 0, 2, and 4 weeks and then monthly. At the dose of 10 mg/kg half-life is 13 days, ranging from 8 to 25 days. Pharmacokinetic analysis revealed a trend toward higher clearance of the drug with increasing body weight.

Mechanism of action

At the beginning, the block of the T cells activation was experimented to act directly on CD28. Unfortunately, the administration of an antibody against CD28 in healthy volunteers evoked a cytokine storm associated with multiorgan failure (104). Thus, efforts have been focused on enhancing the inhibitory action of CTLA-4.

Abatacept is a selective modulator of the CD80/86-CD28 co-stimulatory signal, essential for activation of T cells. It blocks specific binding of the CD80/CD86 receptor in antigen presenting cells to CD28 on T cells, inhibiting the transmission of a second signal of the immune response, and producing a negative signal on T cell activation (101-103).

Approved indications

Rheumatoid arthritis: The use of abatacept for the treatment of RA is approved for patients with moderate/severe disease activity that do not respond to treatment with conventional DMARDs or anti-TNF. Abatacept can be administered in combination with DMARDs. In Table 5 the RCT evaluating the efficacy of abatacept in RA patients are reported (105-108).

Clinical data show a significant efficacy of abatacept in reducing joint inflammation and progression of structural

damage (105). Some patients respond to the drug in 2-4 weeks, but most of them require 12-16 weeks (109). The association of abatacept and MTX determine an improvement of signs and symptoms, physical function and quality of life after one year of treatment with a health maintenance for over 2 years. In addition, radiographic progression shows a further reduction after 2 years of follow-up (110).

Adverse events

Abatacept is generally well tolerated. The increased risk of serious infections in patients treated with the drug was similar if compared with those treated with other biological agents. However, in patients with chronic obstructive pulmonary diseases an increased risk of developing severe infections of the lower airways in conjunction with seasonal exacerbations has been documented. All patients participating in the trials were screened for LTBI and positive patients were treated with abatacept after receiving specific treatment. Moreover, patients should be screened for viral hepatitis before starting the treatment (111). An epidemiological overview has not shown an increased risk of developing malignancies in the patients treated with abatacept (112). The drug exhibits low levels of immunogenicity and the anti-drug antibody response has been reported in less than 3% of patients. However, no appearance of new autoimmune diseases was registered (112).

B-cell-depleting therapy

B cell alterations have been described in several autoimmune diseases, including RA and SLE (113). B cells behave as antigen presenting cells, stimulating the activation and proliferation of T cells. In addition, the synovium of patients with RA contains a large number of plasma cells producing rheumatoid factor (RF) (114). In turn, RF provides a self-perpetuating stimulus for B cells, while the immune complexes RF-Fc receptors induce the synthesis of pro-inflammatory molecules by macrophages, such as TNF (115).

In SLE, the defective tolerance causes the accumulation of a large number of autoreactive B cells producing autoantibodies. In addition, SLE patients exhibit alterations in the B cells homeostasis that result in a lack of naïve B cells and expansion of peripheral blood plasmablasts (116, 117). The maturation of B cells occurs through different stages characterized by a broad spectrum of surface markers. Therefore, there are several potential candidates on which it is possible to act in order to block the function of B cells. The easiest method to obtain a reduction in the number of B cells is to use mAbs directed against surface markers such as CD19, CD20, and CD22. These mAbs bind to the antigens and eliminate the target cells by triggering apoptosis, complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC). Moreover, to reduce the number of B cells, mAbs may also target cytokines involved in their maturation. Among these, the most studied are B lymphocyte stimulator (BLyS) and A Proliferation-Inducing Ligand (APRIL). Rituximab is a chimeric mouse/human mAb that targets CD20, a molecule expressed by more than 95% of the B cells. In fact, the CD20 is found on the surface of immature forms, but not on stem cells and pre-B or plasma cells (116, 117).

Mechanism of action

Rituximab, blocking the CD20, leads to the removal of intermediate stages of B cells. The treatment outcome is a transient but complete depletion of B cells in the blood and a partial depletion of B cells in the bone marrow and synovial tissue. The aim in depleting B cells is to diminish their differentiation into plasma cells and therefore decrease the production of autoantibodies. In 1997, the US Food and Drug Administration (FDA) approved rituximab for the treatment of low grade non-Hodgkin's B cell lymphomas. About ten years later it was approved for the treatment of RA. In RA patients rituximab is administered as two 1 g intravenous doses (given with 100 mg methylprednisolone or equivalent) separated by an interval of 2 weeks.

Approved Indications

Rheumatoid Arthritis: The use of rituximab has been approved in combination with MTX for the treatment of

Table 5. Main randomized controlled trials testing the efficacy of abatacept in RA patients.

Study	Treatment	Treatment duration	Outcomes
ATTAIN, Genovese (105)	Abatacept vs placebo	6 months	ACR response, HAQ
ATTEST, Schiff (106)	Abatacept + MTX vs IFX + MTX vs MTX	6 months	ACR response, EULAR response, HAQ, DAS28, safety
AIM, Kremer (107)	Abatacept + MTX vs MTX	1 year	ACR response, DAS28, HAQ, SF-36, radiographic progression (Genant-modified Sharp score)
ASSURE, Weinblatt (108)	Abatacept + DMARDs (including other biologics) vs DMARDs	1 year	Safety

Legend: RA: rheumatoid arthritis; ACR: American College of Rheumatology; HAQ: Health Assessment Questionnaire; IFX: infliximab; SF-36: Short Form-36; DMARDs: disease modifying anti-rheumatic drugs

patients affected by moderate/severe RA, resistant or intolerant to at least one TNF antagonist (118). In Table 6 the main studies evaluating the efficacy of rituximab in RA patients are reported (118-121). Several trials, performed on patients who had not responded to TNF antagonists, demonstrated a better clinical response in patients treated with rituximab compared with patients treated with another TNF inhibitor (120).

It has been shown that the use of rituximab in combination with MTX is more effective than monotherapy in reducing the inflammatory activity and increasing the functionality and quality of life. The duration of response to a single cycle of rituximab is approximately 6 months. A better response has been demonstrated in patients with positivity for RF and anticitrullinated protein antibodies (ACPA) (118). These patients seem to benefit from a second cycle of rituximab treatment (122).

Adverse events

An increased incidence of bacterial infections in patients treated with rituximab has been registered, as in the case of other biological agents. Available data do not suggest the need for TB screening before starting treatment, while the drug is contraindicated in patients with HBV infection, because cases of fatal viral reactivation have been reported in the literature (123, 124). A few cases of progressive multifocal leukoencephalopathy (PML) have been reported in RA patients treated with rituximab, but the possible explanation remains unknown (111). The most common adverse event is represented by infusion reactions, especially during the first infusion, which may be minimized pretreating the patients with intravenous glucocorticoids, along with acetaminophen and diphenhydramine. Moreover, cases of psoriasis and vasculitis have been described, as with other biological agents (125), while there is no evidence to support an increased risk of malignancies.

Off-label use

Since 2000, rituximab was used to treat SLE patients refractory to conventional treatment producing convincing results in many case series and in uncontrolled trials (126, 127). SLE is a chronic inflammatory disorder with a multifactorial etiology, in which genetic and environmental factors interact in the disease susceptibility (128). The disease is characterized by the production of a wide range of autoantibodies (129-134). SLE mainly affects women in their reproductive age and every organ and/or system can be involved in the pathological process. Moreover, SLE shows heterogenic clinical manifestations (135-141). Several clinical manifestations could be associated with the presence of antiphospholipid antibodies (142-150).

Lu et al. evaluated the efficacy and the safety of rituximab in a cohort of 50 SLE patients resistant to conventional treatment (126). After a 6-months follow-up, a complete remission was achieved by 89% of patients. In those patients who responded to treatment, further analysis showed that clinical improvement appeared to occur across all organ systems of the BILAG disease activity index (126). The Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) and the European Consensus Lupus Activity Measurement (ECLAM) showed a significant decrease after 6 months in a cohort of 23 patients. In the same study, the safety profile of rituximab was evaluated compared with a group of RA patients treated with rituximab because refractory to anti-TNF treatment. While the efficacy was similar in both groups, the safety profile was different, since the infusion-related reactions were significantly more frequent in RA respect to SLE patients (151). These results were in contrast with the findings from the EXPLORER study, a randomized, double-blind, placebo-controlled, phase II/III trial, demonstrating the absence of significant difference between patients who received rituximab and those receiving placebo (152, 153).

Table 6. Main randomized controlled trials testing the efficacy of rituximab in RA patients.

Study	Treatment	Treatment duration	Outcomes
IMAGE, Tak (119)	RTX 2 x 500 mg + MTX vs RTX 2 x 1000 mg + MTX vs MTX	52 weeks	ACR response, EULAR response, DAS28, HAQ, radiographic progression (Genant-modified Sharp)
SERENE, Emery (118)	RTX 2 x 500 mg + MTX vs RTX 2 x 1000 mg + MTX vs MTX	48 weeks	ACR response, EULAR response, DAS28, HAQ, FACIT-F, SF-36, safety
MIRROR, Rubbert-Roth (120)	3 regimens comprising 2 courses of RTX: 2 x 500 and 2 x 500 mg; 2 x 500 and 2 x 1000 mg (dose escalation); and 2 x 1000 and 2 x 1000 mg	48 weeks	ACR response, DAS28, EULAR response, SF-36, FACIT-F, HAQ, safety
SUNRISE, Mease (121)	After receiving 1 course of open-label RTX (2 x 1000 mg), patients were randomized to receive an additional course of RTX or placebo	48 weeks	ACR response, DAS28, HAQ, CRP/ESR levels, EULAR response, safety

Legend: RA: rheumatoid arthritis; RTX: rituximab; MTX: methotrexate; ACR: American College of Rheumatology; EULAR: European League Against Rheumatism; DAS: disease activity score; HAQ: Health Assessment Questionnaire; FACIT-F: Functional Assessment of Chronic Illness Therapy-Fatigue; SF-36: Short-Form 36; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

New drugs targeting B cells

Belimumab: It is a mAb constituted of a human recombinant IgG. It acts by binding BLyS protein and preventing the interaction with the B cell activating factor (BAFF) receptor. In this way, the activation, differentiation and long-term survival of mature B cells, the secretion of autoantibodies and the activation of the T cells are inhibited (154). The role of BLyS in the pathogenesis of some autoimmune diseases has been suggested by several evidences. High levels of BLyS and circulating heterotrimers formed by BLyS/APRIL have been reported in patients affected by RA, SLE, and SS (154-156). In addition, BLyS and APRIL polymorphisms have been implicated in susceptibility to SLE development (157). Belimumab seems to act more effectively on newly activated B cells rather than memory B cells or plasma cells (154).

The pharmacokinetic profile is similar to that of the intravenous Ig and other recombinant human mAbs. The drug is given as an intravenous infusion at a dose of 10 mg/kg every 2 weeks for the first 3 doses, then every 4 weeks. Belimumab is generally well tolerated. The most frequent adverse events reported in phase II and III clinical trials on SLE patients were headache, upper respiratory tract infections, lower urinary tract infections, diarrhea, nausea, hypotension and fatigue (158-160). The use of belimumab in the treatment of SLE has been approved by the FDA in 2011. Two International Studies (BLISS) were conducted to evaluate the efficacy and safety of belimumab in patients with SLE (159, 160). The two studies differed primarily in the geographic regions in which they were conducted. The first (BLISS-76) was conducted in Europe and North America, the second (BLISS-52) in Eastern Europe, Latin America, Asia, and Pacific. Both have enrolled more than 800 patients and applied the SLE Responder Index (SRI) as the primary efficacy endpoint at 52 weeks (159). BLISS-76 was carried out up to 76 weeks. Patients who had active lupus nephritis or severe active involvement of the central nervous system were excluded from the study. At week 24, patients achieved a good therapeutic response and improvements in physician's global assessment score (159). Regarding the effects on serological features, the treatment with belimumab was able to bring back to normal the levels of C3 and C4 complement fractions, reduce hypergammaglobulinemia and anti-dsDNA antibody concentrations (159, 160). The results from the phase III BLISS-52 have shown that a significant number of patients receiving belimumab 1 mg/kg or 10 mg/kg achieved a reduction greater than 4 points in the SELENA-SLEDAI score at 52 weeks compared with patients receiving placebo. The BLISS-52 is the first successful study on the efficacy of a biological agent in SLE (160).

Atacicept: This is a recombinant fusion protein comprising the extracellular domain of the TACI (Transmembrane Activator and CAML Interactor) receptor joined to a human IgG1 Fc domain. It functions mainly by blocking the interaction between BLyS/APRIL with their receptor TACI expressed on mature B cells, plasma cells and activated T cells (161). It has been ascertained that the long-lived B cell progenitors cannot survive when deprived of signals from BLyS. Conversely, the pool of memory cells does not undergo any reduction and, as a consequence, the humoral response to pathogens is not altered (117). Atacicept also inhibits the survival of long-lived plasmacells directly involved in the pathogenesis of RA, SLE and SS (161, 162). In SLE, a study has shown a dose-dependent reductions in B cells and immunoglobulin levels, without any changes in T cells, natural killer cells or monocytes following treatment with atacicept (161).

Epratuzumab: This is a humanized mAb formed by an IgG1 directed against CD22. CD22 is a lectin-like member of the Ig superfamily solely expressed by mature B cells. Its function is to modulate the B cell receptor and signal transduction through CD19, and participates in mediating signals for survival (163). Although the precise role of CD22 has not yet clarified, recent studies suggest that blocking its action with the use of a mAb could lead to a reduction of peripheral B cells and inhibition of the B proliferation in SLE patients, negatively modulating B cell migration and the expression of adhesion molecules (164).

Anti-IFN

Type I IFN seems to play a central role in the pathogenesis of SLE and is therefore a potential therapeutic target. The alterations involve primarily IFNα, maybe due to the presence of specific genetic polymorphisms that affect the production of type I IFN, its activities and serum concentrations (165). The immune complexes found in blood of patients with SLE contain anti-dsDNA antibodies and nucleic acids and it has been shown that these immune complexes are able to stimulate the action of IFN. In the blood and tissues of patients with SLE numerous IFN-producing cells, and an increase of IFN mRNA and of the IFN itself, were also found (166).

Sifalimumab: This is a fully human IgG1κ mAb that binds to IFNα with high affinity and prevents IFNα signaling through its receptor. The phase I study on patients with (SLE) demonstrated a good safety profile that supports further clinical development (167).

Conclusions

The biological drugs have revolutionized the management of the patients affected by chronic inflammatory rheumatic diseases, allowing a better prognosis and the achievement of clinical remission in a significant percentage of patients. These drugs target different molecules directly involved in the pathogenesis of several diseases, such as RA, PsA, AS and SLE. In Table 7 we reported the approved indications of the available biological drugs according to the European Medicine Agency (www.ema.europa.eu). New biological drugs are now under investigation.

Table 7. Approved indications of the available biologic drugs according to European Medicine Agency (AIFA).

Drug	Approved indications			
	RA	PsA	AS	SLE
Abatacept	x			
Adalimumab	x	x	x	
Belimumab				x
Certolizumab	x			
Etanercept	x	x	x	
Golimumab	x	x	x	
Infliximab	x	x	x	
Rituximab	x			
Tocilizumab	x			

Legend: RA: rheumatoid arthritis, PsA: psoriatic arthritis, AS: ankylosing spondylitis, SLE: systemic lupus erythematosus.

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