Monoclonal Antibodies as Therapeutic Agents for Inflammatory Diseases

Jéssica Bairos, Ana P.M. Tavares, Emanuel V. Capela* and Mara G. Freire

CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal.

Abstract: Inflammation is a physiological process caused when an agent (chemical, biological or physical) transcends the primary defense barrier of an organism, playing a central role in the fight against those pathogens, setting a series of biological reactions to restore the integrity of such organism. Uncontrolled amplification of these events may lead to undesirable pathological manifestations such as cancer, diabetes, and cardiovascular, neurological, and chronic inflammatory diseases. Monoclonal antibodies (mAbs) were first described in 1975, and since then they have proven to be relevant therapeutic agents in a myriad of diseases. The US Food and Drug Administration (FDA) has already approved more than 90 mAbs for the treatment of several diseases, from which approximately 46% were specifically approved for the treatment of inflammatory diseases, for instance rheumatoid arthritis, Crohn's disease, ulcerative colitis, psoriasis, psoriatic arthritis and palmoplantar pustulosis. This chapter aims to provide an overview on the inflammation process and main biochemical mechanisms, a vision on the current state of the art of the mAbs-based biopharmaceuticals market, and describes the mAbs products already approved by regulatory agencies as powerful therapeutic agents for inflammatory diseases, while highlighting the advantages of these biopharmaceuticals and fomenting their widespread use as recurrent therapies.

Keywords: Inflammation, inflammatory diseases, biochemical mechanisms, biopharmaceuticals, biopharmaceuticals market, therapeutic agents, monoclonal antibodies.

INTRODUCTION

Inflammation consists in the natural protective response of body to injury. It occurs when an agent (chemical, physical or biological) transcends the primary defense barrier of the organism [1,2]. It plays a central role in the fight against pathogens and can set biochemical reactions to restore homeostasis through the activation of specific components, which act through the destruction or isolation of the aggressor agent [3,4]. Inflammation can be manifested as an acute process, comprising three main events: i) increased blood flow; ii) development of edema, and iii) migration of leukocytes to the inflammatory focus [2]. Uncontrolled amplification of these events may lead to a chronic process, which is of long-term and associated with the presence of lymphocytes fibrosis and tissue necrosis [5,6]. This phenomenon causes undesirable pathological manifestations such as cancer, diabetes, and cardiovascular, neurological, and chronic inflammatory diseases [5,6]. Therefore, this type of diseases' progression fostered the search for effective alternative therapies, which is a crucial objective to be achieved in the coming years.

In recent decades, technological advances in bioprocess engineering have increased the interest in the development of alternative therapies for inflammation treatment, particularly recurring to biopharmaceuticals [7]. Biopharmaceuticals are biological macromolecules or cellular components that can be used in vaccines or as therapeutic agents. They are obtained by biological processes (*in vitro* or *in vivo*), and are extracted from biological sources, for example tissues and organs, microorganisms, fluids of animals, from mammalian cell cultures, insects, and also plants [7]. Main examples comprise recombinant proteins (monoclonal antibodies) and nucleic-acid-based products, which can be applied in the treatment of several inflammatory diseases, for instance in Crohn's disease, ulcerative colitis, rheumatoid arthritis, psoriasis, psoriatic arthritis and palmoplantar pustulosis [8]. Among them, monoclonal antibodies are the most used biopharmaceuticals, representing 53% of all biopharmaceutical industry [9].

Monoclonal antibodies (mAbs) were firstly described by Köhler and Milstein in 1975 [10], and since then, they have become the new backbone of the pharmaceutical industry since they have exquisite target selectivity and specificity [10]. mAbs offer the most promising prospects for new therapeutic approaches for inflammatory diseases [11,12]. The major successful applications of mAbs is in autoimmune and inflammatory conditions, such as Crohn's disease, ulcerative colitis, rheumatoid arthritis, spondyloarthropathies, juvenile arthritis, psoriasis and psoriatic arthritis [13]. Furthermore, as a wide range of mAb-based agents target several cytokines, chemokines, adhesion molecules, receptors and various types of cells, it is expected that these therapeutic "magic bullets" will greatly expand in the future, while providing better personalized treatment for a wide range of diseases.

In this chapter, the most important aspects and main biochemical mechanisms of the inflammation process are overviewed, followed by a current review on the mAbs-based biopharmaceuticals market and approved mAbs product/therapies for inflammatory diseases. The action mechanisms and features of some relevant mAbs are also discussed, highlighting the advantages of mAbs-based therapies, while envisaging the adoption and widespread use of these biopharmaceuticals as recurrent treatments in the near future.

MONOCLONAL ANTIBODIES

Structure and properties of antibodies

Antibodies, usually referred as immunoglobulins (Igs), are glycoproteins found in plasma and extracellular fluids [14]. They are essential components in the immune-humoral system of all vertebrates [14], being the line of defense of the immune system [15]. They are produced naturally by specific plasma cells, namely the B lymphocytes, in response to the exposure to "foreign" molecules or other antigens [14,15]. In their composition they present one or more regions, the paratopes, which recognize and bind to the epitopes of the antigen. This molecular recognition allows the neutralization and/or elimination of the antigen, allowing the organism to protect itself against the action of microorganisms and other harmful species, such as foreign proteins [16–18], carbohydrates [18], peptides [16], bacteria [16,18], viruses [16], fungus [17] or even cancer cells [17]. As a result, an effective immune response takes place, often involving the production of a vast array of antibodies that are structurally similar, yet unique, thus enabling the multiple epitope binding onto a given antigen [16,19].

Independently of their specificity, all antibodies are heterodimer proteins structurally composed with four polypeptides chains – two identical heavy chains (H) and two similar light chains (L), in a “Y”-like shape form (Figure 1) [14]. Disulphide bonds and non-covalent bonds held together these chains by the "hinge" region, that provides stability and flexibility to the antibody. Furthermore, all four polypeptide chains contain variable regions (V), which presents considerable variations in their amino-acid composition, and where the antigen binds [16]. The constant regions (C), located at the carboxyl terminal region, is specific for effector functions [16]. The antibody chains are further divided into L and H sections – each L chain has a variable domain (VL) and a constant domain (CL), while each H chain has one variable domain (VH) and three constants domains (CH1, CH2, CH3) [14,16,20], as shown in **Figure 1.**

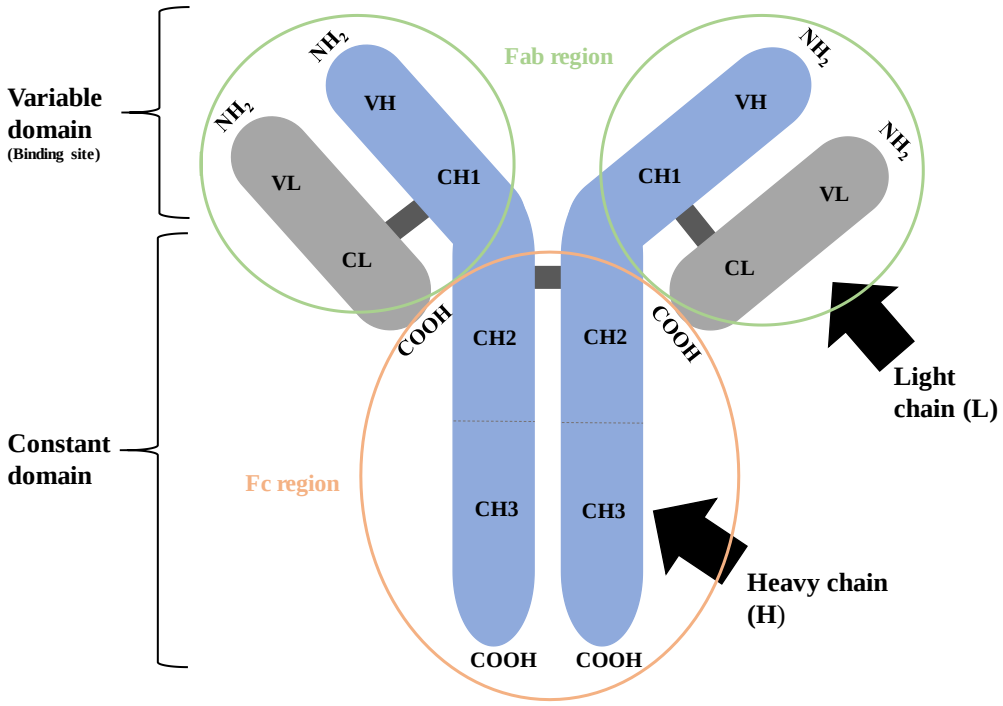


Figure 1: Basic structure of antibodies. It consists in a "Y"-shaped molecule composed of two heavy (blue) and light (grey) chains. Each one of these chains contains multiple constant (C) and one variable (V) regions linked by disulphide bonds. The antigen-binding site is located on the N-terminal region, whereas their effector domain reside on the C-terminal region.

The antibodies may undergo proteolytic digestion, giving rise to new antibody fragments – Fab (**F**ragment **a**ntigen **b**inding) and Fc (**F**ragment **c**rystalline) [14]. Some enzymes can be highlighted in this topic: papain, for example, digests the antibodies into two Fab fragments and one Fc fragment, whilst pepsin cleaves the antibody below the disulphide bridge, generating an Fc fragment and a divalent F(ab') fragment [14]. The Fab fragment of the antibody contains the specific antigen-binding domain, and the Fc fragment is responsible for the effector properties, such as activation of natural killer cells, activation of the classical pathway of the complement system and phagocytosis of the antigen [14].

Antibodies can operate through several mechanisms to neutralize potential harmful effects by "foreign" organisms. Once an antibody's Fab region binds to the antigen, through complementarity determining regions (CDRs), its interaction with other ligands is blocked, and an agonist signal is emitted that triggers several signaling cascades [21]. Depending on the Fc region, these "magic bullets" can act through two main mechanisms: (i) antibody-dependent cell-mediated cytotoxicity (ADCC), mediated by natural killer (NK) cells, macrophages, neutrophils or eosinophils, in which effector cells trigger phagocytosis or lysis of the target cell, whose surface was already covered by specific antibodies [21,22]; or (ii) complement-dependent cytotoxicity (CDC), where antibodies eliminate the pathogens triggered by the complement cascade on the cell membrane [21,23].

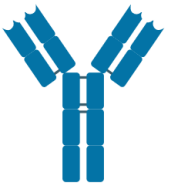
Five classes of antibodies can be found in mammals, performing different functions (detailed in **Table 1)** according to each foreign body type that they find: IgA, IgD, IgE, IgG and IgM

[14]. IgG and IgA can be further divided into subclasses, referred as isotypes, due to polymorphisms in constant regions in the heavy chains. IgG can be split into four different subclasses - IgG1, IgG2, IgG3, and IgG4 - each presenting its own biological properties, whereas IgA can be split into IgA1 and IgA2 [24]. Among them, IgG is the most abundant bloodstream antibody. IgG comprises 80% of all the Igs and 20% of the total proteins in human serum, achieving a concentration of 10-25 $mg \cdot mL^{-1}$ [25,26]. This class of antibodies has an isoelectric point (pI) comprised between 5.5 and 9.5 [27] and a molecular weight (MW) of approximately 150 kDa [15]. Moreover, IgG is composed of two identical heavy chains (55 kDa each) and two light chains (25 kDa each) [14].

Considering the origin of the antibodies, they can be classified into monoclonal and polyclonal. The differences between them define the limitations of use of each type of antibody. Most antigens are extremely complex, exhibiting numerous epitopes that are recognized by several lymphocytes [14,15]. Each lymphocyte is activated in order to proliferate and differentiate into plasma cells, and the resulting antibody is polyclonal. In fact, serum is an excellent source of polyclonal antibodies, they are produced from a mixture of lymphocytes and recognize multiple epitopes on the same antigen, being able to bind to different substances [14]. Polyclonal antibodies can be produced in large quantities, since it is relatively fast and cheap and do not require specific technical competences, when compared to the production of mAbs [14,28]. This capability helps to amplify the signal produced by the target protein, since the antibody binds to more than one epitope, resulting in a more robust detection. Furthermore, polyclonal antibodies are more tolerant of eventual changes that occur in a reduced number of epitopes, such as polymorphisms, glycosylation, or slight denaturation. In addition, these antibodies show greater stability over a wider range of pH and salt concentration, while mAbs are much more sensitive to small changes in both parameters due to the lack of variability between them [14,28]. mAbs are highly specific and obtained from a single hybrid B lymphocyte cell and recognize not only the same antigen, but also the same epitope [14,28]. Since they recognize and interact with a specific substance, they are excellent alternatives in given therapeutic purposes and in the evaluation of changes in molecular conformations, protein-protein interactions, glycosylation/phosphorylation states and identification of unique members of protein families [14,28].

In what concerns their mode of action, mAbs can act through direct or indirect effects. Directly, the antibody binds to cell surface receptors, growth factors, membrane bound proteins or circulating proteins, modulating the cells [29]. Indirect effects occur when mAbs bind to target cells and stimulate the recruitment of effector cells (natural killer cells and monocytes/macrophages) by promoting cellular cytotoxicity or phagocytosis. Indirect effects can also be achieved through the conjugation of mAbs with toxins, drugs, cytokines or radioisotopes, providing the specialized delivery of the diagnostic or therapeutic agents [29]. This allows for standardization of tests and, additionally, reduces background noise and cross-reactivity [14,28]. Given their advantage in different fields and as therapeutic agents in given diseases, mAbs are the focus of the current chapter.

Table 1: Characteristics, function, and structures of mammal's antibodies.

	Serum Concentration ($mg \cdot mL^{-1}$) [26,30]	Functions	Structure
IgA	3-3.5	Found in mucosal areas, preventing its colonization with pathogens [31].	
IgD	Trace amounts	Less defined functions.	
IgE	Trace amounts	Elimination of parasites. It causes release of histamine, thus being involved in allergies [32].	
IgG	10-25	Main antibody in the secondary response. Participates in opsonization and activation of the complement system [33].	
IgM	1-2	Removes pathogens in the early stages of (humoral) B cell-mediated immunity before there is enough levels of IgG [34].	

Relevance and applications of monoclonal antibodies

The pharmaceutical industry and medicine have increasingly sought new methods to produce drugs that are more sensitive, specific and with a lower incidence of risks and adverse effects [7]. In the last twenty years, there have been relevant developments by the introduction of biotherapeutics [8]. Biopharmaceuticals have an active principle that is obtained through the

use of microorganisms or genetically modified cell; examples are hormones, recombinantly expressed cytokines, blood factors, replacement enzymes or antibodies [8]. Advances in molecular biology and in mAbs technology and engineering allowed their introduction into the currently important group of biopharmaceuticals [8]. In particular, mAbs differ from traditional drugs since they present low variability, biological production and unique antigenic specificity to targeted pathogens [7].

mAbs were first originated by the hybridoma technology developed by Köhler and Milstein [10], in 1975, to produce mAbs with unique target selectivity. This technique starts by injecting in a mammal (e.g.: murine) a known antigen or mixture of antigens that incites an immune response. Then a B cell produces Abs that bind to the injected antigen and posteriorly these isolated B cells are fused with immortal myeloma cells to produce an hybrid cell line – a hybridoma [29]. The hybrid cells can be grown in culture with a selection medium that only allows the immortalized hybrids to survive. Each hybridoma produces only one specific mAb, which is monitored, so that the clone with the desired specificity can be selected and expanded. The products obtained from these individual clones are mAbs specific for a single epitope on an antigen or antigen mixture [35,36]. Despite the development in mouse-mAbs production, their use as therapeutic agents also present some drawbacks in humans. In addition to having a short half-life in serum compared to human IgG, they can cause allergic reactions, induce anti-drug mAbs, promote insufficient activation of functions human effectors and, if used continuously in humans, stimulate an immunological reaction - a human antibody response against those of murine origin, called **H**uman **A**nti-**M**urine **A**ntibodies (HAMAs) response [37].

Aiming at solving the drawbacks associated with mAbs obtained from hybridoma, several efforts have been done along the years to humanize antibodies through genetic engineering. To produce these humanized/fully human mAbs, new techniques have been developed, namely recombinant DNA technology, which consist on genetically manipulating the genes responsible for the production of the desired mouse mAbs, making its amino acid structure very close to the structure of human amino acids, while maintaining the specificity of mAbs [38]. Recombinant mAbs (also referred as second-generation mAbs) are created by the immortality of the immunoglobulin-producing genes, rather than being produced by the immortality of the mAb-producing cell. By recombinant DNA techniques it is possible to include a human character to mouse antibodies by changing the genes encoding the immunoglobulin chains, thus changing the structure and function of these mAbs [38]. Firstly, a chimeric antibody is generated by the introduction of the mouse sequences for the variable region in a human antibody, allowing to decrease the risk of immunogenicity. Then, humanized mAbs are developed by substituting the rodent sequences for human sequences, except those sequences within the antigen-binding complementarity determining regions [29]. Finally, fully human mAbs can be produced by phage-display platforms where, *in vitro*, antibodies are expressed on the surface of a phage used to infect *Escherichia coli* that replicate and produced the desired mAb, as well as *in vivo* by producing transgenic mice,

expressing human variable domains coupled to hybridoma technology, allowing the production of fully human mAbs with low immunogenic potential [39,40].

Although highly relevant, the evolution of the described upstream technologies only partially solved one of the downsides of Abs – immunogenicity. For example, the administration of infliximab (chimeric mAb, brand name – Remicade) in patients with Crohn's disease, makes them develop **H**uman **A**nti-**C**himeric **A**ntibodies (HACAs) that can bind to the therapeutic antibodies, restraining its half-life and clinical effectiveness, as well as infusion-related anaphylaxis in some patients [41]. The development of mAbs from murine to humanized/fully human partially solved the immunogenicity issues associated to therapeutics. Nonetheless, the development of **H**uman **A**nti-**H**uman **A**bs (HAHAs) can be also an obstacle, causing the same problems as HACAs [41]. The advantage of fully human mAbs, compared to other classes, is the fact that they allow multiple administrations without causing allergies or immunogenic reactions, being of total safety for chronically or immunosuppressed patients. Hereupon, once the desired hybridoma has been generated, mAbs can be produced through a constant and renewable source, allowing an incessant and reproducible number of antibodies [14].

Monoclonal antibodies therapeutics market

The biopharmaceutical market has been growing steadily since 1982, the year in which the first biopharmaceutical, a recombinant human insulin (Humulin) produced by *Escherichia coli*, was approved for the treatment of diabetes by the Food and Drug Administration (FDA) [42]. Later on, in 1986, a human protein tissue plasminogen activator (tPA) [43] became the first therapeutic protein from mammalian cells to obtain approval in the market [44].

Following these two hallmarks, antibodies have proven to be of high value in the biopharmaceuticals market, both economically and in the improvement of therapeutic efficiency. Their success as therapeutics depends on their efficacy, safety and pharmacoeconomic issues. Despite the effectiveness and safety of mAbs for human administration, especially when they have a high degree of purity and retain specific activities, the access to this type of therapy has been fraught by high manufacturing costs. In clinical practice, the therapeutic agents should be selected not only based on their efficacy, but also by considering their safety and economic limits. Some drugs have been withdrawn along the years from the market, due to safety concerns, whereas the clinical use of other agents is not deemed to be cost-effective [13,45]. Monoclonal antibodies are the main products in the global biopharmaceutical market, being assessed at approximately US$115.2 billion in 2018 and expected to generate revenues of US$300 billion by 2025 [44]. Their use in human therapy has been increasing in the market, representing 53% of all biopharmaceutical industry [9].

The use of antibodies for the treatment of several pathologies begun in 1890, when Behring and Kitasato [29] found the ability of small doses of diphtheria or tetanus toxin to provide immunity between animals via serum (later explained by the presence of antibodies in the matrix). Though, it was only in the early 1960s that the structural features of the antibodies

were exposed, and it was only in the next decade that methods for producing mAbs were discovered, as mentioned above [29]. Likewise, in 1986, muromonab-CD3 (Orthoclone OKT3) was the first mAb approved by FDA and European Medicines Agency (EMA) for the treatment of kidney transplant rejection [29], whereas the first therapeutic mAb (infliximab) for the treatment of inflammatory diseases was approved in 1998 [41]. mAbs can be used either as a monotherapy or in parallel with other standard therapeutic methods, particularly if the disease under treatment is willful to therapy using exclusively conventional techniques. This combination provides an exclusive prospect for the treatment of painful and incurable diseases [13,43].

There are currently 90 therapeutic mAbs approved by FDA for the treatment of several diseases (data acquisition in September 2020) and approximately 570 antibody therapeutics at various clinical phases [46]. From these, approximately 46% correspond to mAbs approved for the treatment of inflammatory diseases (as mentioned before, examples are Crohn's disease, rheumatoid arthritis, ulcerative colitis, palmoplantar pustulosis (PPP), psoriasis, psoriatic arthritis [13,47]. In general, these therapeutic mAbs for inflammatory diseases can act by blocking ligand-receptor interactions targeting the receptor [41,45,48,49] or negatively modulating the cell surface receptor expression that can also be indirectly achieved by ligand targeting [41]. The success of mAbs in inflammatory conditions marks their fast evolution with over 150 different mAbs currently under clinical trials for further approval by the FDA and EMA [45].

INFLAMMATION OVERVIEW

Inflammation occurs in vascularized connective tissues, involving the capillary beds, plasma, circulating cells, sensory neurons, and cellular and extracellular constituents of this type of tissue. It is a physiological process caused when a chemical, physical and/or biological invader agent transcends the primary defense barrier of the organism, the epithelial and/or endothelial layer, and its specialized structures [1,2]. Its role is to restore the homeostasis of the damaged tissue through the activation of the specific components that generate the effector cells and their products (cytokines and antibodies) and non-specific components of immunity, which act through the destruction or isolation of the aggressor agent, involving the action of phagocytic cells and mediators as well as their migration to the lesion site [3,4]. Inflammation can manifest as an acute or chronic process. During the acute inflammatory process there are several events mediated by cellular and vascular components that induce morphological and biochemical changes [5]. Among them, three main events are highlighted: i) increased caliber of arterioles, capillaries and venules, which cause increased blood flow; ii) exudation of plasma proteins, complement factors and antibodies, which contribute for the development of edema; and iii) migration of leukocytes from the intravascular space to the inflammatory focus [2]. Together, these events characterize the classic signs of inflammation: flushing, heat, tumor, pain, and loss of function [50]. On the other hand, the chronic

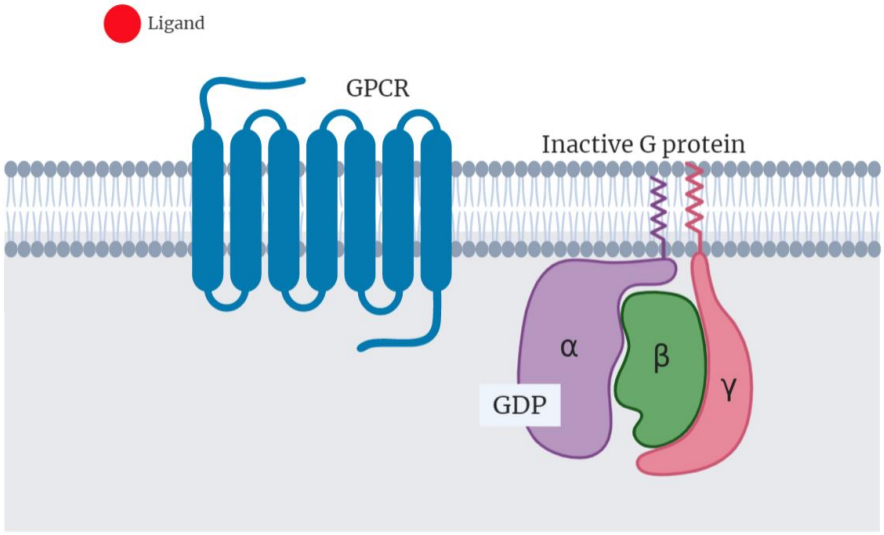
inflammatory process is of long-term and associated with the presence of lymphocytes and macrophages, proliferation of blood vessels, fibrosis and tissue necrosis [5,6].

Before an injury, epithelial tissue separates the external environment or a body cavity from the underlying and more delicate connective tissue and body organs. The connective tissue is nurtured by blood vessels and mast cells. Both the inflammatory response and wound healing occur simultaneously, but consisting in separate processes that begin immediately after the injury [51]. After aggression, the inflammatory response is triggered and the accumulation of cells from the immune system (leukocytes, macrophages and lymphocytes) occurs, secreting various cytokines and chemokines [51]. Leukocytes express several types of receptors in their surface that recognize external stimulus and release activating signals. Those receptors can be G-protein-coupled receptors (GPCRs) [52], adhesion receptors (selectins and integrins) [53], pattern recognition receptors (PRRs) [54], Fc-receptors [55] and cytokine receptors [56,57].

G-protein-coupled receptors

GPCRs, known as G protein-linked receptors (GPLR) or serpentine receptors (**Figure 2**), are coupled to G proteins. They belong to a large family of protein receptors that distinguish molecules outside the cell and activate internal signal transduction pathways (the cyclic adenosine monophosphate (cAMP) signal and the phosphatidylinositol signal) and finally, cellular responses [52,58,59]. They are found in neutrophils and macrophages and participate in host defense and inflammation. These include formyl-peptide receptors [52,58,59] that sense bacterial products and tissue injury, receptors for leukotriene B4, platelet activating factor and complement fragment [59–62], as well as α-chemokines and β-chemokines receptors [63–65]. All of them activate in a strong way the chemotactic migration of leukocytes and trigger other responses, including the production of reactive oxygen species (ROS), exocytosis of intracellular granules and vesicles, and are able to augment the responses of leukocytes to subsequent stimulation by other agonists [66]. GPCRs are formed by seven transmembrane domains, with the amino terminal at the extracellular medium and the carboxyl terminal in the intracellular medium and interact with G proteins. In the moment that an external signaling molecule binds to a GPCR, a conformational change in the GPCR occurs. This change then triggers the interaction between the GPCR and a nearby G protein [52,58,59]. The bond promotes a conformational change in the intracellular domain of the receptor, which allows its interaction with a second protein (stimulatory G protein). The occupied receptor causes replacement of guanosine diphosphate (GDP) bound to the Gα subunit by guanosine triphosphate (GTP), activating the Gα subunit. This subunit dissociates from the Gβγ dimer and an intracellular signaling cascades is started [52,58,59]. It results in the activation of adenylate cyclase, small GTPases, phospholipases and kinases, eventually being capable to control the expression of genes that are involved in survival, proliferation and differentiation [52,58,59].

Figure 2:



Representation of the G-protein-coupled receptors (GPCR) and G protein subunits (GDP: G_{alpha} (α), G_{beta} (β) and G_{gamma} (γ)). The G protein is attached to the inside of the cell membrane but is able to move along it. When GDP is attached to the G protein, it is inactive. The ligand activates the GPCR, inducing a conformational change in the receptor that allows him to function as a guanine nucleotide exchange factor (GEF) that exchanges GDP for GTP – thus turning "on" the GPCR.

Adhesion receptors

Adhesion receptors (**Figure 3)** are responsible for the initial stabilized binding of leukocytes to the blood vessel wall and their succeeding transendothelial migration to the perivascular tissue, either during normal recirculation and or the inflammation process [53]. Most of them belong to the four protein families: selectins, integrins, cadherins and the Ig superfamily (IgCAMs). The two major groups involved in the inflammation process are selectins and integrins. The first are single-chain transmembrane glycoproteins that are able to recognize carbohydrate moieties and mediate transient interactions between leukocytes and the vessel wall [53]. Selectins and selectin ligands are mandatory for the rolling phase of the leukocyte adhesion and transmigration cascade [53,67]. On the other hand, integrins can be defined as heterodimeric transmembrane glycoproteins that are present on all mammalian cells [68]. The most important integrins expressed on leukocytes belong to the β2 integrin [69]. Lymphocyte function-associated antigen 1 (LFA-1) is expressed on all circulating leukocytes while macrophage-1 antigen (Mac-1) is primarily expressed on myeloid cells such as neutrophils, monocytes, and macrophages. LFA-1 and Mac-1 bind to endothelial intercellular Adhesion Molecule-1 (ICAM-1) and are involved in different phases of leukocyte adhesion and transendothelial migration [70].

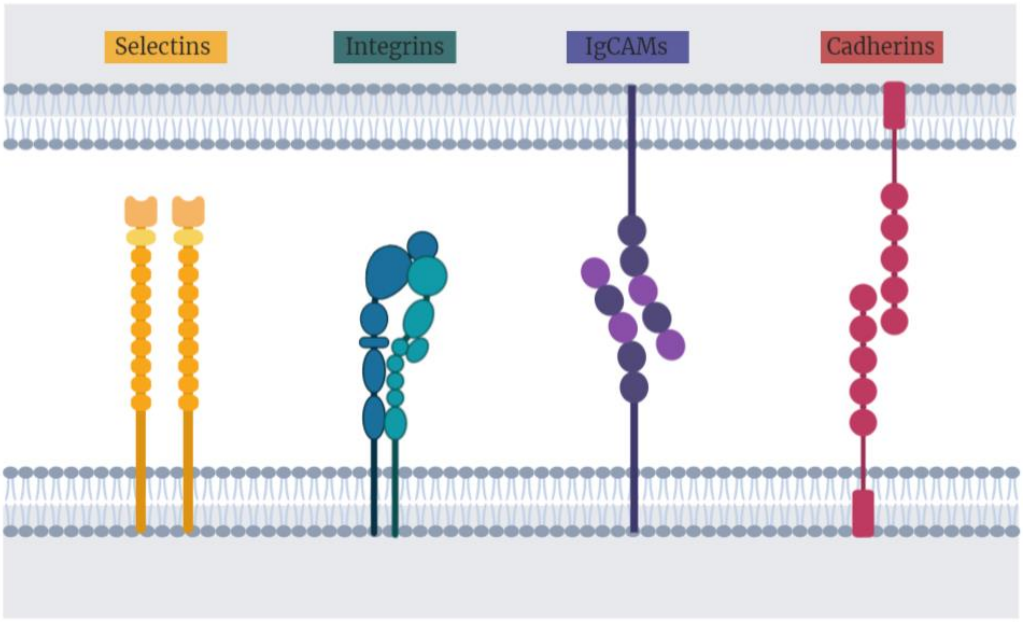
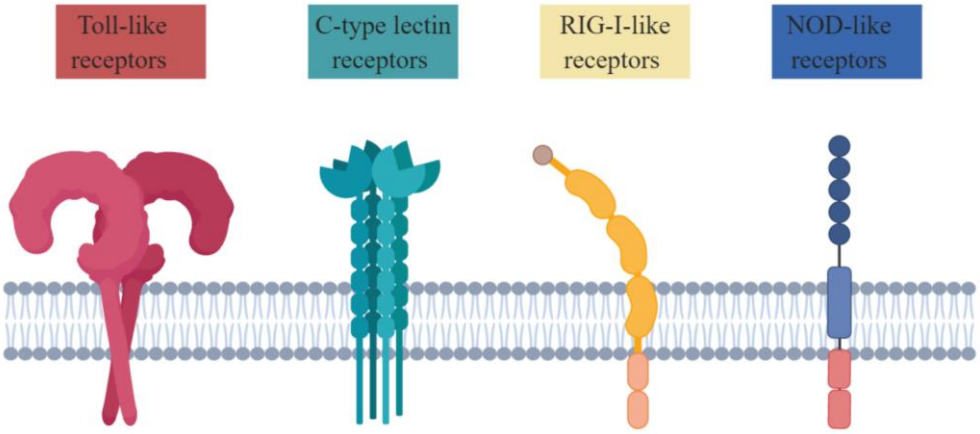


Figure 3: Different types of cell adhesion receptors, form the left to right: selectin-mediated cell adhesion receptor (sMcAr); integrin-mediated cell adhesion receptor (iMcAr) immunoglobulin superfamily-mediated cell adhesion receptor (IgMcAr) and cadherin-mediated cell adhesion receptor (cMcAr).

Pattern recognition receptors

Pattern recognition receptors (PRRs) (**Figure 4**) are important in the innate immune response by recognizing the pathogen associated molecular patterns (PAMPs) and the endogenous molecules released from damaged cells, called damage associated molecular patterns (DAMPs) [54,71]. Those pathogens can be bacteria, viruses, parasites, fungi, and protozoa. They are expressed in macrophages, dendritic cells and in various nonprofessional immune cells [54,71]. The PRRs are either localized on the cell surface, to perceive extracellular pathogens, or within the endosomes. These receptors are involved in triggering pro-inflammatory signaling pathways, stimulating phagocytic responses or binding to microbes as secreted proteins [54,71]. They can also be classified into four different classes of PRR families: transmembrane proteins like toll-like receptors (TLRs); C-type lectin receptors (CLRs); cytoplasmic proteins like retinoic acid-inducible gene (RIG)-I-like receptors (RLRs) and NOD-like receptors (NLRs) [54]. The TLRs function through kinases to stimulate the production of microbicidal substances and cytokines by leukocytes [72]. These proteins have an important relationship with the interleukin- 1 (IL-1) receptor. Several studies [54,66,71] have demonstrated that these proteins activate the nuclear factor-κB (NF-kB) and mitogen-activated protein kinase (MAPK) pathway. Furthermore, they regulate the expression of cytokines through various adaptors such as TIR domain-containing adaptor protein (TIRAP), Myeloid differentiation primary response gene 88 (MyD88), TIR-domain-containing adaptor inducing IFNβ (TRIF), Trif-related adaptor molecule (TRAM), and Sterile-α and Armadillo motif-containing protein (SARM). The activation of the NF-kB pathway initiates an immune adaptive response by the production of inflammatory cytokines such as IL-1, IL-6, IL-8,

TNF-α, IL-12 [54,66,71]. The CLRs through the recognition of carbohydrates interact with some microorganisms, for instance, viruses, fungi, and bacteria. CLRs are also involved in the



modulation of the innate immune response. These recognitions allow the internalization of the pathogen, subsequent degradation and then antigen presentation. CLRs can stimulate the production of proinflammatory cytokines or inhibit TLR-mediated immune complexes. Most of these receptors signal through an immunoreceptor tyrosine-based activation motifs (ITAM)-based mechanism like Fc-receptors or through the activation of protein kinases or phosphatases. CLR-induced signal transduction seems to mainly activate or modulate NF-κB functions [54,66,73,74]. RLRs are a family of RNA helicases that recognize genomic RNA of dsRNA viruses and dsRNA generated as the replication intermediate of ssRNA viruses [54,66,71]. After detection of a viral infection, RIG-I and MDA5 cooperate with the adaptor IFN-b-promoter stimulator 1 (IPS-1 also called VISA, CARDIF and MAVS) via CARD-CARD interactions. IPS-1 activates the release of cytokines and the IKK-related kinase, which activates IRF3/IRF7, resulting in the transcription of type I interferons. IPS-1 also activates NF-κB through recruitment of TRADD, FADD, caspase-8, and caspase-10 [54,66,71]. The NLRs are cytoplasmic sensors of PAMPs, DAMPs and danger signals that lead to transcriptional changes or activate cytokine-processing caspases. They can work together with Toll receptors and regulate the inflammatory and apoptotic response. The protein receptor NOD1 and NOD2 which port CARDs domain, activate NF-κB and MAP-kinase pathways via an adapter (RIP2/RICK). NF-κB then activates the expression of inflammatory cytokines [54,66].

Figure 4: Major pattern recognition receptors (PRRs) presented from the left to right: Toll-like receptors (TLRs), C-type lectin receptors (CLRs), cytoplasmic proteins like Retinoic acid-inducible gene (RIG)-I-like receptors (RLRs) and NOD-like receptors (NLRs).

Fc-receptors

Fc-receptors (**Figure 5**) are proteins found on the surface of B lymphocytes, follicular dendritic cells, NK cells, macrophages, neutrophils, eosinophils, basophils, and mast cells

[55]. They have the ability to bind to antibodies in their Fc region and are involved in the recognition of Ig-opsonized pathogens, participating as well in immune complex-mediated inflammatory processes [55]. These receptors promote phagocytic or cytotoxic cells to destroy microbes or cells which were infected by antibody-mediated phagocytosis or ACDD. Some viruses (e.g. flaviviruses) use Fc receptors to help them infect cells, by a mechanism known as enhancement of antibody-dependent infection [55]. The most important Fc-receptors in neutrophils are the low-affinity Fcγ-receptors [75]. These receptors present important roles in immune complex mediated activation of neutrophils. The activation of leukocytes by immune complexes requires synergistic ligation of both FcγRIIA and FcγRIIIB [76]. They also express the high-affinity FcγRI molecule [77,78] and FcαRI, which can mediate IgA-induced inflammatory processes, tumor cell killing [79,80], and may participate in allergic responses [81,82] or as pathogenic factors in certain infectious diseases [83].

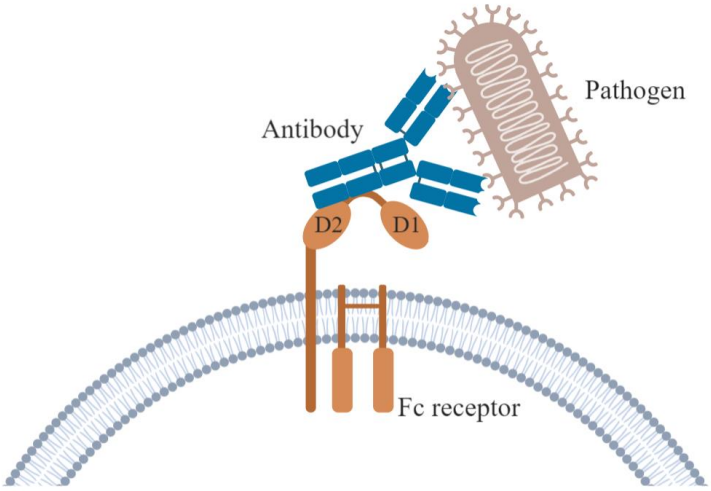


Figure 5: Schematic illustration of an Fc receptor and its interaction with an Ab-coated microbial pathogen. Fc receptor binds to the antibodies in their Fc region, triggering the recognition of Ig-opsonized pathogens.

Cytokine receptors

Cytokine receptors (**Figure 6**) are cell surface glycoproteins that when bonded to cytokines have their signal transduced. These receptors permit the communication between the cells and the extracellular environment, responding to signals produced in the surrounding area in the body [56]. Therefore, the first binding of cytokines to their receptors is a crucial event that is fast, in low concentrations, generally irreversible and leads to intracellular changes, resulting in a biological response [56]. They comprise six group members, based on their three-dimensional structure, namely type I, type II, Ig superfamily, tumor necrosis factor (TNF) receptor family, chemokine receptor and transforming growth factor β (TGF- β) receptor family. Conventional cytokine receptors are grouped into type I and type II. Those types of receptors are involved in a few neutrophil functions. Type I receptors consists in transmembrane receptors expressed on the surface of cells, recognizing and responding to cytokines with four α-helical strands [56]. G-CSF and GM-CSF guide the differentiation, survival and activation of neutrophils [56]. IL-4, IL-6, and IL-15 are involved as well in

activation of neutrophils and in the coordination of the inflammatory response. Type II are similar to type I cytokine receptors, except they do not possess the signature sequence of common amino acid motif. IFNα/β delay apoptosis of neutrophils [57], whereas IFNγ which is secreted by NK cells reacting to antigens and activated T lymphocytes during adaptive immune responses. IFN-γ is a major macrophage activating cytokine [84]. IL-10 presents an inhibitory effect on various functional responses of neutrophils, namely chemokine and cytokine production [85]. Type I and type II cytokine receptors trigger the activation of the JAK-STAT pathway [86–88], Src-family kinases [89–92], the PI3-kinase-Akt pathway [90,92–94], the ERK and p38 MAPK [95,96], and the inhibitory SOCS molecules [97–99]. Ig superfamily are involved in the recognition, binding, or adhesion processes of cells. They all possess a domain known as an immunoglobulin domain or fold. Included in this group are molecules involved in the presentation of antigen to lymphocytes, cell adhesion molecules, cell surface antigen receptors, co-receptors and co-stimulatory molecules of the immune system [56,100]. TNF receptors are characterized by the ability to bind tumor necrosis factors (TNFs) via an extracellular cysteine-rich domain. They are engaged in apoptosis and inflammation phenomena, but also participate in other signal transduction pathways, such as proliferation, survival and differentiation [100]. The chemokine receptor interacts with a type of cytokine called a chemokine. Each has a rhodopsin-like 7-transmembrane (7TM) structure that allows to couple to G-protein for signal transduction within a cell, making them members of the large protein family of GPCR. After interaction with their specific chemokine ligands, the chemokine receptors trigger a flux in intracellular calcium (Ca^{2+}) ions. This event causes cell responses, including the onset of a process known as chemotaxis that traffics the cell to a desired location within the organism [100]. TGF-β receptors are serine/threonine kinase involved in cell growth, cell differentiation, apoptosis and cellular homeostasis [56,57]. TGFβ ligands bind to a type II receptor, which recruits and phosphorylates the type I receptor. Then it phosphorylates the receptor-regulated SMADs (R-SMADs) which bind the coSMAD SMAD. The complex R-SMAD/coSMAD accrue in the nucleus where they join in the regulation of target gene expression, acting as a transcription factor [56,57].

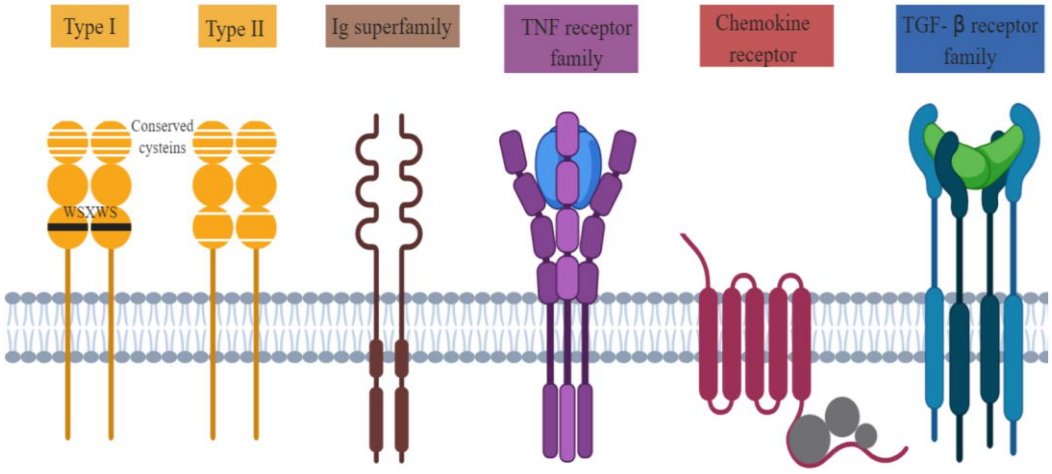


Figure 6: Types of cytokine receptors: type I and type II families possess extracellular fibronectin like domains, only differing in the WSXWS motif present in the type I, that are not present in type II receptors. The Ig superfamily shares extracellular regions structural homology with immunoglobulin domains. The TNF receptor family has cysteine-rich motifs in their extracellular regions able to bind ligands. Chemokine receptor are G protein coupled receptors and TGF- β receptor family are Serine/threonine kinase receptors.

After accumulation, mast cells are stimulated by the chemokine alarm chemicals to release histamine. Adhesion of neutrophils (first leukocyte to respond) is mediated by adhesion molecules, whose expression is enhanced by secreted proteins known as cytokines [101,102]. These are secreted by cells in response to microorganisms and other harmful agents, ensuring that neutrophils are recruited into the tissues. The initial interactions of bearing are mediated by selectins [103,104], which are divided into three types: one expressed in leukocytes (L-selectin), one in the endothelium (E-selectin) and one in platelets (P-selectin). Its expression is regulated by cytokines produced in response to inflammation and injury. Leukocytes (neutrophils and monocytes) express L-selectin at their surface, and as a result they roll along the endothelial surface. This rolling is regulated by TNF [105] and IL-1 [106], which induce the endothelial expression of integrin ligands, especially vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM- 1). The expression of integrin ligands induced by the cytokines and the activation of the integrins in the leukocytes, results in a firm attachment to the endothelium [68]. Chemokines act on adherent leukocytes and cause changes in their shape, allowing migration to the interstitial tissue fluid [107]. From the receptor in the surface of the leukocytes, are initiated signals, that result in activation of a second messenger that increased cytosolic Ca^{2+}, activate enzymes such as protein kinase C and phospholipase A_2 and induce polymerization of actin, ensuing in increased quantities in direction of the cell border and localization of myosin filaments. In the intercellular junctions there are adhesion molecules called platelet endothelial cell adhesion molecule (PECAM-1) or cluster of differentiation 31 (CD31) that aid in transmigration [101,108]. The leukocytes migrate in the direction of the locally produced chemoattractant gradient. After crossing the endothelium, the leukocytes leave the circulation and migrate to the tissues towards the lesion site by a process called chemotaxis [109]. When leukocytes reach the site of inflammation, they are activated to perform their functions, recognition of aggressive agents, which release

signals and these signals activate the leukocytes to ingest and destroy the hostile agents and amplify the inflammatory reaction [110].

The functional responses which are the most important for the destruction of microbes and other harmful agents are phagocytosis and intracellular killing. Phagocytosis involves three sequential steps: i) recognition and binding of the particle to be ingested by the leukocyte; ii) its intake, with subsequent formation of the phagocytic vacuole; and iii) death or degradation of the ingested material [111,112]. Phagocytosis depends on the polymerization of actin filaments and is increased when the microbes are opsonized by specific proteins, opsonin, for which phagocytes express high affinity receptors [113]. After binding of the microorganisms to the receptors, extensions of the cytoplasm flow around them and the plasma membrane closes in a vesicle, called the phagosome. It fuses with the lysosome, resulting in discharge of the bead content into the phagolysosome [113]. The last step in the removal of infectious agents and necrotic cells is death and degradation within neutrophils and macrophages. The microbial death is carried out by ROS and reactive nitrogen species (RNS) [111,112]. The generation of ROS is catalyzed by the action of NADPH oxidase, that oxidizes NADPH and reduces oxygen to the superoxide anion ($O_2^{\bullet-}$). $O_2^{\bullet-}$ is converted to hydrogen peroxide (H_2O_2), whichever cannot efficiently destroy microbes. However, H_2O_2 can be converted to the hydroxyl radical ($OH^{\bullet}$), or through the enzyme myeloperoxidase (MPO), converted to hypochlorite ($OCl^{\bullet}$), both potent antimicrobial agents that destroy microbes by halogenation or oxidation of proteins and lipids [114]. NO also participates in microbial death. It reacts with $O_2^{\bullet-}$ to generate the peroxynitrite radical ($ONOO^{\bullet}$). These free radicals attack and damage the lipids, proteins and nucleic acids of microbes [115]. The elimination of microbes and dead cells activated leukocytes have other roles in the defense of the host. After this “cleansing”, macrophages produce growth factors that stimulate endothelial cell proliferation, fibroblasts and collagen synthesis, that remodel connective tissues, allowing healing and the end of the inflammatory process [116].

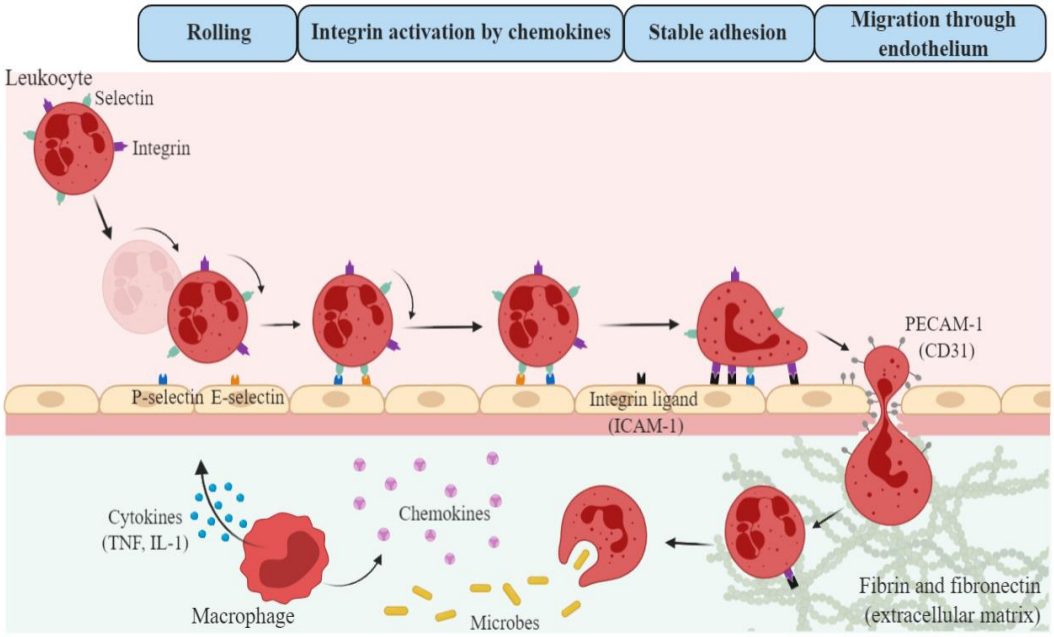


Figure 7: Firstly, leukocytes roll and then become activated, adhering to the endothelium. Following occurs their penetration in the basement membrane and migration towards the chemoattractants released at the source of the injury. Different molecules have important roles in different phases – selectin in the scroll; chemokines activated neutrophils; integrins in firm adhesion and CD31 (PECAM-1) in transmigration. Adapted from Kumar *et al*. [110].

Inflammation plays a central role in the fight against pathogens and can set biological reactions to restore the integrity of the organism. Hysterical amplification of the events may lead to undesirable pathological manifestations such as neoplastic transformations due to the oxidation of DNA, cancer, diabetes, and cardiovascular, neurological, and chronic inflammatory diseases. Therefore, it is necessary to limit the inflammatory process by eliminating the cellular infiltrate and its potentially toxic products [5,6].

MONOCLONAL ANTIBODY-BASED THERAPIES FOR THE TREATMENT OF INFLAMMATION

Interleukin-8 and interleukin-6

There are more than 40 different chemokines that can be classified according to the location of the cysteine residues at the amine terminus [117]. One of the most widely studied chemokine is interleukin-8 (IL-8), also called chemokine ligand 8 (CXCL8) (**Figure 8**) [118]. Based on a chain of biochemical reactions, IL-8 is produced by leukocytes and epithelial and endothelial cells [118]. IL-8 is initially produced as a 99 amino acid precursor peptide, and then undergoes cleavage to create various active IL-8 isoforms. The peptide containing 72

amino acids, possess a molecular weight of 8.4 kDa and a isoelectric point > 8.5, is the mature form secreted by macrophages [35,119]. IL-8 is a key mediator associated with inflammation, mediating the recruitment and activation of neutrophils through complex signaling mechanisms and extracellular adhesion molecules [35,120]. Its receptors are found on the surface membrane of various cells of the immune system. The most important are the G protein-coupled receptors, which after binding to IL-8 activate the intracellular signaling cascades and triggers a conformational change, resulting in the activation of G protein [35,120]. G protein subunits stimulate phosphatidylinositol 4-phosphate kinase (PIPK) which in turn synthesizes phosphatidylinositol 4,5-bisphosphate (PIP_2), being the source of inositol trisphosphate (IP_3) and phosphatidylinositol (3,4,5)-trisphosphate (PIP_3). IP_3 leads to the release of Ca^{2+} that induces chemotaxis, oxidative burst, exocytosis and eventually the release of more inflammatory mediators [35,120]. PIP_3 activates ras/raf/MAPK pathways, inducing the expression of adhesion molecules, such as integrin, fundamental for chemotaxis [35,120].

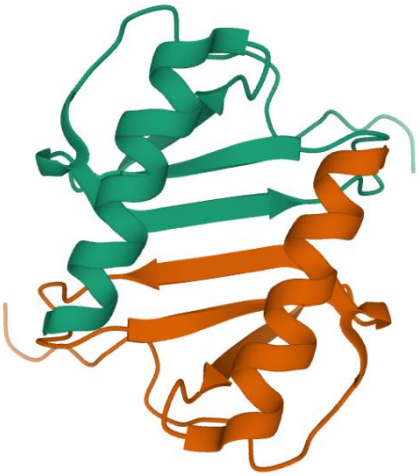


Figure 8: 3D structure of interleukin-8 (Image from the Protein Data Bank website (http://rcsb.org/pdb) of PDB ID 1IL8 [121].

One highly relevant pro-inflammatory cytokine is interleukin-6 (IL-6) (**Figure 9**). It was originally discovered in 1986 by Hirano *et al.* [122], as a T cell–derived B cell stimulatory factor-2, promoting Ig synthesis by activated B-cells. Its productions is associated with many cell types, including monocytes, macrophages, lymphocytes, endothelial cells and fibroblasts, and can be stimulated by interleukin-1 (IL-1) and TNF [123,124]. Human IL-6 consist of a polypeptide cytokine with a four–α-helix structure and is composed of 212 amino acids, including a 28-amino-acid signal peptide, and its gene has been mapped to chromosome 7p21. Even though the core protein possess 20 kDa, glycosylation is responsible

for the size of 21 – 26 kDa of natural IL-6 [123,124]. IL-6 family cytokines are a group of cytokines consisting of IL-6, oncostatin M (OSM), leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), cardiotropin-1 (CT-1), cardiotrophin-like cytokine (CLC), neuropoietin (NP), IL-11, IL-27, and IL-31. In the receptor complexes, all of them contain a common receptor signal transducer subunit (gp130), except IL-31 [124].

IL-6 is a pleiotropic cytokine which play a part in the short-term defense against infection or injury, warning the immune system against the source of inflammation [125]. Upon being secreted during an acute inflammatory response, IL-6 forms a protein complex with its specific α-receptor (IL-6R) and the ubiquitously expressed 130 kDa transmembrane protein mentioned before – gp130 – which encourages the transition from neutrophil to monocyte in inflammation, initiating the signal transduction [126]. This binding induces the homodimerization of gp130 that triggers cellular events, including activation of the JAK/STAT3 pathway. Then, STAT3 and SHP2 are phosphorylated and activated by the activated JAK. SHP2 links the cytokine receptor to the Ras/MAPK pathway and it also links the Grb2-SOS complex and Gab1 to gp130 [123,126–128]. Then, the phosphorylated STAT3 forms a dimer and translocate into the nucleus in order to activate the transcription of genes comprising STAT3 response elements. STAT3 induces as well the expression of the suppressor of cytokine signaling 1 and 3, that binds to phosphorylated JAK and gp130, respectively, leading to the cessation of IL-6 signaling via a negative feedback loop [123,126–128]. Nevertheless, IL-6 can also signal through its soluble form, sIL-6R, which is present in human serum. After binding IL-6 to sIL-6R, the complex binds to gp130, consequently stimulating cells that do not express transmembrane IL-6R, for example smooth and endothelial muscle cells. This form of IL-6 signaling is known as IL-6 trans signaling, while transmembrane IL-6R signaling is known as classical IL-6 signaling [127].

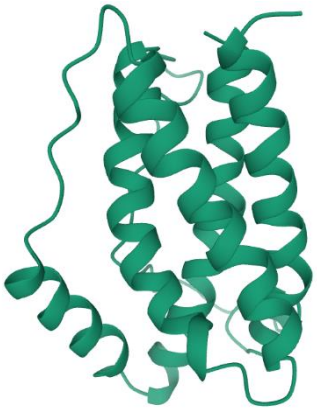


Figure 9: 3D structure of interleukin-6 (Image from the Protein Data Bank website (http://rcsb.org/pdb) of PDB ID 1IL6 [129].

Anti-interleukin-8 and anti-interleukin-6 mAbs

Interleukin-8 induces morphologic change of neutrophils and release activated substances under ischemic and hypoxic conditions. On the other hand, it is an important neutrophilic

granulocyte chemotactic regulator and endorses infiltration of neutrophils into the vascular wall, causing its destruction and hyperplasia [120,130]. IL-8 accelerates proliferation, migration and incrassation of smooth muscle cells, exacerbating vasospasm and vasogenic edema [120,130]. Generation of IL-8 can be expected upon infection, trauma, ischemia and other disturbances of tissues, since IL-1 and TNF levels are increased [119]. Studies presented by Alcorn *et al.* [131] reported high IL-8 levels in asthma, obstructive lung disease and acute respiratory distress syndrome. Also, as the inflammatory response represents a major component of the tumor microenvironment, being responsible for the mediation of the biological communication network and the molecular signaling flow, it is important to observe the levels of this interleukin in this situation. A study by Abdollahi *et al.* [132] showed that overexpression of IL-8 and/or its receptors increases tumor growth and angiogenesis, a critical step for tumor metastasis [132].

Taking into account the overproduction of IL-8 and aiming its reduction, it was revealed in 1989 by a study in rheumatoid synovial membrane cultures that the use of anti-tumor necrosis factor (anti-TNF) mAbs inhibited the local production of proinflammatory mediators, such as interleukins (IL-1, IL-6, IL-8) [13,45]. During the inflammatory process, the activation of cells took place after binding to IL-8 receptors, which are expressed in immune system cells such as neutrophils, monocytes, endothelial cells, astrocytes and microglia [47,133]. The accumulation of active neutrophils in the injured areas and the overproduction of IL-8 can lead to chronic inflammatory conditions [47,133]. The overproduction of IL-8 has been proposed to significantly contribute to all these pathologies, characterized by the accumulation of activated neutrophils in injured areas [47,133].

Tests using mAbs against IL-8 in animal models with acute inflammation showed inhibition or reduction of neutrophil function and partially solved inflammation [47,133]. Several studies [47,133,134] have shown that anti-IL-8 significantly reduces neutrophil infiltration in the early stage of the inflammation event and that anti-IL-8 treatment also reduced redness and sagging of the joints and prevented membrane damage synovial [47,133]. In the study conducted by Yang *et al.* [135], it was found that among all fully human IgG2κ anti-IL-8 mAbs studied, K4.3 and K2.2 (derived from Xeno-Mouse strains) blocked IL-8 binding to human neutrophils, as well as the activation of neutrophils and chemotaxis of neutrophils. Skov *et al.* [47] provided an *in vivo* proof of concept of the application of an anti-human IL-8 antibody (HuMab 10F8) for the treatment of inflammatory diseases, such as palmoplantar pustulosis [47]. Mahler *et al.* [136] demonstrated that the use of anti-IL-8 antibody (ABX-IL8) in the treatment of chronic obstructive pulmonary disease was well tolerated and safe, once the neutralization of IL-8 led to small but significant reducing of the severity of dyspnea, the major symptom of this disease, which evidences a reduction in the number of neutrophils in the blood [136]. Investigations have also been conducted with HIV, where Guha *et al.* [136] found that anti-IL-8 restored 38% and 22% of neuronal death. As there are inflammation situations in cancer, this IL-8 mAbs is starting to be used in the treatment of cancer, being however under development [137]. An important finding in this field was reported by Huang *et al.* [137], showing promising results with the use of this therapy in a

melanoma case. The results revealed that ABX-IL8 does not inhibit the proliferation of melanoma cells *in vitro*, but increases the number of apoptotic tumor cells and significantly suppressed tumorigenicity *in vivo* [137]. Using a more complex model system of metastatic primary tumors, histochemical analysis confirmed that anti-IL-8 therapy results in a significant reduction of Matrix Metalloproteinase-9-neutrophils, associated with reduced levels of angiogenesis [138].

Interleukin-6 elicits acute phase reactions, but also the development of specific cellular and humoral immune responses, namely end-stage B cell differentiation, immunoglobulin secretion and T cell activation. It is produced at low levels, but there is an increase in its expression in the presence of inflammation or trauma [123–125]. After its production in the inflammatory region, it reaches the liver through the bloodstream. Following occurs a rapid induction of the secretion of vascular endothelium growth factor (VEGF), leading to the increased growth of blood vessels and vascular permeability in inflammation. It also induces high concentrations of SAA for a long time, that leads to the development of amyloid A amyloidosis – a severe complication of chronic inflammatory diseases [123–125].

The dysregulated overproduction of IL-6 has been implicated in the development of several autoimmune and chronic inflammatory diseases (rheumatoid arthritis, systemic juvenile arthritis and Crohn's disease) [139]. This association of IL-6 with inflammatory diseases was first shown in 1988 in a case of rheumatoid arthritis, where the patients were detected with high levels in synovial fluids [140]. Subsequent studies [123] have shown that this dysregulation also occurred in swollen lymph nodes of Castleman's disease, myeloma cells and peripheral blood cells or involved tissues in various other chronic inflammatory diseases. Consequently, it is extremely important to regulate the magnitude and duration of the response of this unregulated IL-6 production. One of the first approved mAbs for the treatment of inflammatory diseases was Tocilizumab (trade name, Actemra) in 2010 [141]. This humanized anti-IL-6R mAb binds both soluble and transmembrane receptor of IL-6 (IL-6R), blocking the action of IL-6 without increasing its half-life [142]. Clinical studies [139,142–144] demonstrated its outstanding efficacy for the treatment of rheumatoid arthritis and Castleman's disease. For the treatment of rheumatoid arthritis, tocilizumab was quite effective with > 80% and > 30% of patients achieving ACR20 and ACR50 responses (clinical response parameter established by the American College of Rheumatology (ACR)), respectively [142]. Tocilizumab can be used as a monotherapy or in combination with disease-modifying antirheumatic drugs, and it has significantly suppressed the disease activity and radiographically detected progression of joint deformity, allowing to improve daily functional activity [139,142–144]. Later, in 2014, Siltuximab (trade name Sylvant) [48], anti-IL-6 chimeric (made from human and mouse proteins) mAb, was approved for the treatment of patients with Castleman's disease who do not have human immunodeficiency virus (HIV) or human herpesvirus-8 (HHV-8). Van Rhee *et al.* [145] studied the assess treatment of long-term safety and activity of Siltuximab for 6 years, and concluded that in 97% of patients idiopathic multicentric Castleman disease was controlled, supporting the use of this anti-IL-6 mAb as a first-line therapeutic in this disease. Sarilumab (trade name Kevzara, 2017), a humanized anti-IL-6R mAb, is able to inhibit IL-6 signaling, which otherwise would upregulate the release of rheumatoid arthritis-related factors from hepatocytes [146]. Sarilumab was approved by FDA for the treatment of moderately to

severely active rheumatoid arthritis in people who do not respond or tolerate more conventional treatments. The biopharmaceutical can be used alone or in combination with methotrexate or other disease-modifying antirheumatic drugs [146]. Fleischmann *et al.* [147] showed a reduction of the absolute neutrophil counts (<1000 cells.mm^{-3}) for 13% and 15% of patients treated with combination therapy and only Sarilumab, allowing to confirm its long-term safety profile. Also, a humanized anti-IL-6R mAb, Satralizumab (trade name Enspryng), was the last one to be approved so far, in 2020. It is indicated for the treatment of neuromyelitis optica spectrum disorder (NMOSD) in adults with a particular antibody (anti-aquaporin-4 positive) [148]. Traboulsee *et al.*[149] proved that the monotherapy with Satralizumab reduces the risk of relapse by 55% for all NMOSD patients and by 74% in anti-aquaporin-4 positive-IgG seropositive patients, suggesting it as a safe and effective alternative for all NMOSD patients. Yet, there are many clinical studies underway on anti-IL-6 mAbs, such as Sirukumab (CNTO136), Olokizumab (CP6038), Elsilimomab (BE-8), Clazakizumab (BMS945429) and MEDI5117 which are in different phases of clinical trials to ascertain their efficacy and safety [150].

All previously reported studies here discussed suggest that anti-IL-8 and anti-IL-6 mAb-based therapy, if proven effective in clinical trials, could be used to treat a broad spectrum of disorders, with particular interest for inflammatory diseases.

Role of anti- IL-6 mAbs in the Severe Acute Respiratory Syndrome-2

Inflammation is a stereotyped response; so, it is reflected as a mechanism of innate immunity, as compared to adaptive immunity that is specific for each pathogen. The inflammation response turns out to be transversal to several diseases, not only the inflammatory ones, but also infectious diseases for example. Infections are a frequent cause of inflammation and are caused by an infectious agent. The inflammation they cause depends on the type of infectious agent and the location of the organism where it is installed [151]. A known example of this is the coronavirus (CoVs), which contain a large group of viruses and is one of the main pathogens directed mainly to the human respiratory system. In the past decade, two new viruses have revealed to be highly pathogenic infectious agents for humans, causing potentially lethal infections. They are the coronaviruses responsible for Severe Acute Respiratory Syndrome (SARS-CoV) and the coronaviruses of the Middle East Respiratory Syndrome (MERS-CoV) [152–154]. Genetic analysis carried out to date have shown that the new CoVs belong to the same group that includes SARS-CoV, identified 10 years ago [152–154]. The designation of the new coronavirus as 2019-nCoV has been replaced by SARS-CoV-2, which means that it is the second coronavirus in the SARS group.

The SARS-CoV-2 was first identified in humans in December 2019 in the city of Wuhan, China, causing an infection disease, called Coronavirus Disease 2019 (COVID-19) that had spread worldwide [152–155]. This infection can be asymptomatic, but even in these cases, infected individuals can transmit the virus to other people, especially during the first days

after the infection, when the viral replication in the upper respiratory tract is particularly productive [152–155]. The clinical signs and symptoms that characterize COVID-19 are very diverse, including fever, cough, illness and breathing difficulties, with invasive lesions in lungs that appear after an incubation period that can vary between 2 to 14 days [153]. In more severe cases, the infection can cause pneumonia, SARS, kidney failure and even death [153]. Although the virus can infect people of all ages, it appears to be particularly aggressive for individuals over the age of 65 with co-morbidities (example diabetes, hypertension, liver problems or immunosuppression due to cancer) [152–155].

SARS-CoV-2 is easily transmitted from person to person in two ways, through droplets and aerosols emitted with coughing, sneezing or during conversation at small distances. Its replication (Figure 10) on host cells occurs when the S glycoprotein on the virion binds to cellular receptor angiotensin-converting enzyme 2 (ACE2) and enters the target cells via an endosomal pathway [154,156]. Following the entry, the viral RNA is unveiled in the cytoplasm and some RNA is translated into polyproteins, which are cleaved by proteases. Some of these proteins form a replication complex to produce more RNA. After the production of SARS-CoV-2 structural proteins, nucleocapsids are assembled in the cytoplasm and followed by budding into the lumen of the endoplasmic reticulum (ER)–Golgi intermediate compartment. The virions are then released from the infected cell via exocytosis [154,156].

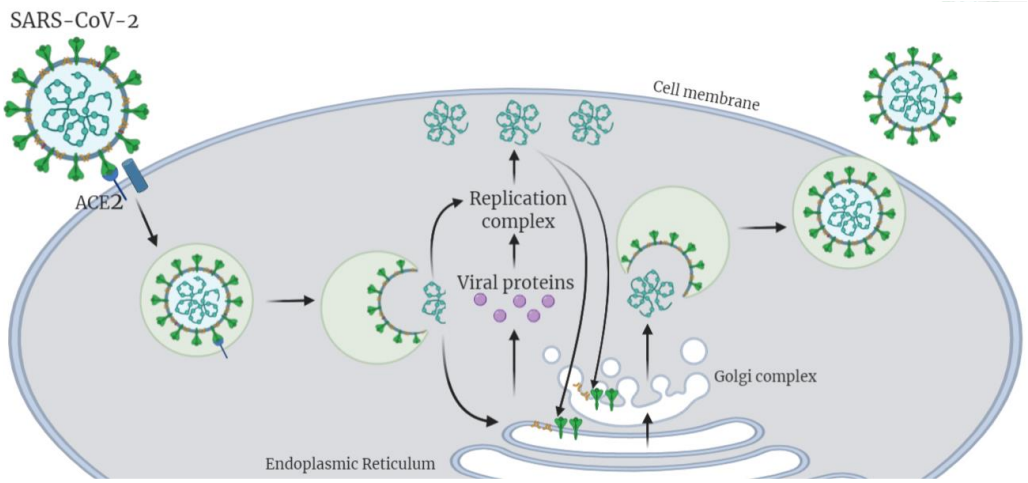


Figure 10: Life cycle of SARS-CoV-2 in the host cells. S glycoprotein on the virion binds to ACE2. The virion then releases its RNA, some being translated into proteins by the cell's machinery. Proteins and RNA are assembled into a new virion in the Golgi complex and released from the cell.

There are several approaches to the treatment of viruses, but is important to recognize that, at the moment, there are few available antiviral treatments. Considering the most recent pandemic, the approaches involved convalescent plasma therapy and antiviral therapies, with the aim of reducing viral replication, which is the main pathogenic mechanism. Another

possible approach is immunomodulatory therapies, which are directed at the inflammatory response that leads to ARDS [157].

According to the pathogenesis of SARS-CoV-2, several studies evidenced that inflammatory cytokines and chemokines, including IL-6, IL-12, induced protein 10 (IP10), monocyte chemoattractant protein-1 (MCP-1) and TNF-α are considerable released in COVID-19 patients [158–160]. *Post-mortem* pathological analysis revealed tissue necrosis, interstitial macrophage and monocyte infiltrations in the lung, heart, and gastrointestinal mucosa [158–160]. These analysis are in accordance with IL-6 overproduction, which leads to an excessive signaling pathway and contribute to organ damage, including the maturation of naive T cells into effector T cells, induction of VEGF expression in epithelial cells, increased vessel permeability and reduced myocardium contractility [158]. Therefore, targeting IL-6 and its receptor (IL6R) using mAbs, such as Tocilizumab, could mitigate cytokine storm-related symptoms in severe COVID-19 patients [161]. In 2017, Tocilizumab was approved by FDA for severe life-threatening cytokine release syndrome [162]. Moreover, Xu et al. [163] observed, with repeated doses of tocilizumab 4 mg·kg Intravenous (maximum dose 400 mg), a recovery of 91% of patients with severe respiratory symptoms. Also with the same dosage, Buonaguro et al. [159] observed that most patients experienced a 75% improvement in the need for lower oxygen, an increase in lymphocyte levels, decreased fever and improvement in chest tightness. Taking these results into account, since March 2020, Tocilizumab has been officially included in the treatment program for COVID-19 (7th edition) of the National Health Commission of China for patients with increased levels of IL-6, extensive opacity of bilateral lung injuries or in critically ill [164].

Based on the exposed it seems quite plausible to affirm the protective role of anti-IL6R mAb in disorders such as COVID-19, and in particular Tocilizumab that demonstrated to be an effective treatment in severe patients. What conveys a lot of hope is that in the past two months in China, clinical treatment with Tocilizumab has shown remarkable efficacy and safety, promoting a certain expectation of benefiting other countries that are currently fighting the pandemic. However, it should be remarked that anti-IL6R may only be controlling the “cytokine storm” with no deleterious effect on virus replication [159,164].

Other recently approved monoclonal antibodies for inflammatory diseases

In December 2019, 79 therapeutic mAbs have been approved by the FDA, and nowadays this value is already higher than 90; still, there is significant growth potential associated to these biopharmaceuticals [146]. During 2019 and 2020, 13 new mAbs started to be commercialized by several known pharmaceutical companies, such as Genentech®, Roche®, Novartis®, Alder BioPharmaceuticals®, Immunomedics®, Chugai Pharmaceutical®, AstraZeneca®, Viela Bio® and MedImmune®, for the treatment of moderate-to-severe plaque psoriasis, diffuse large B-cell lymphoma, osteoporosis, macular degeneration, sickle cell disease, urothelial cancer, HER2+ breast cancer, migraine prevention, multiple myeloma, thyroid eye disease, triple-negative breast cancer and neuromyelitis optica spectrum disorder. Among the 13 approved mAbs, 6 were suggested to be used in the treatment of inflammatory diseases,

such as plaque psoriasis [165], diffuse large B-cell lymphoma [166], Macular degeneration [167] and Thyroid eye disease [168]. In Table 3, the FDA-approved mAbs for the treatment of inflammatory diseases are summarized, being the 6 most recent approved highlighted in bold.

Table 3: FDA-approved mAbs for the treatment of inflammatory diseases.

Antibody	Brand name®	Type	Disease	Approval date	Ref.
Rituximab	Rituxan	Chimeric	Non-Hodgkin lymphoma	1997	[45]
Palivizumab	Synagis	Humanized	Prevention of respiratory syncytial virus infection	1998	[169]
Infliximab	Remicade	Chimeric	Crohn disease	1998	[41]
Alemtuzumab	Lemtrada	Humanized	B-cell chronic lymphocytic leukemia	2001	[170]
Adalimumab	Humira	Fully Human	RA	2002	[41,45]
Ibritumomab tiuxetan	Zevalin	Murine	Non-Hodgkin lymphoma	2002	[171]
Omalizumab	Xolair	Humanized	Asthma	2003	[41]
Ranibizumab	Lucentis	Humanized	Macular degeneration	2006	[172]
Certolizumab-pegol	Cimzia	Humanized	Crohn disease	2008	[41,48]
Golimumab	Simponi	Fully Human	RA, Psoriatic arthritis, and Ankylosing spondylitis	2009	[41,48]
Canakinumab	Ilaris	Fully Human	Cryopyrin-associated periodic syndrome	2009	[41]
Ustekinumab	Stelara	Fully Human	Plaque psoriasis	2009	[41,48,49]

Ofatumumab	Arzerra	Human	Chronic lymphocytic leukemia	2009	[45]
Tocilizumab	Actemra	Humanized	RA	2010	[41,45,48,49]
Belimumab	Benlysta	Fully Human	Systemic lupus erythematosus	2011	[173]
Brentuximab vedotin	Adcetris	Chimeric	Hodgkin lymphoma and systemic anaplastic large cell lymphoma	2011	[174]
Obinutuzumab	Gazyva	Humanized	Chronic lymphocytic leukemia	2013	[175]
Vedolizumab	Entyvio	Humanized	Ulcerative colitis and Crohn disease	2014	[41,49]
Siltuximab	Sylvant	Chimeric	Castleman disease	2014	[48]
Blinatumomab	Blincyto	Murine	Acute lymphoblastic leukemia	2014	[176]
Secukinumab	Cosentyx	Fully Human	Psoriasis	2015	[177]
Mepolizumab	Nucala	Humanized	Severe eosinophilic asthma	2015	[178]
Ixekizumab	Taltz	Humanized	Plaque psoriasis	2016	[48]
Reslizumab	Cinqair	Humanized	Asthma	2016	[179]
Sarilumab	Kevzara	Fully Human	RA	2017	[48]
Guselkumab	Tremfya	Fully Human	Plaque psoriasis	2017	[48]

Ocrelizumab	Ocrevus	Humanized	RA and Systemic lupus erythematosus	2017	[41,45]
Gemtuzumab ozogamicin	Mylotarg	Humanized	Acute lymphoblastic leukemia	2017	[180]
Brodalumab	Siliq	Chimeric	Plaque psoriasis	2017	[181]
Benralizumab	Fasenra	Humanized	Asthma	2017	[182]
Inotuzumab ozogamicin	Besponsa	Humanized	Relapsed or refractory B-cell precursor acute lymphoblastic leukemia	2017	[183]
Moxetumomab pasudotox-tdfk	Lumoxiti	Mouse	Hairy cell leukemia	2018	[184]
Tildrakizumab-asmn	Ilumya	Humanized	Plaque psoriasis	2018	[185]
Ibalizumab-uiyk	Trogarzo	Humanized	HIV	2018	[186]
Caplacizumab	Cablivi	Humanized	Thrombotic thrombocytopenic purpura	2018	[187]
Risankizumab	**Skyrizi**	**Humanized**	**Moderate-to-severe Plaque psoriasis**	**2019**	**[165]**
Polatuzumab vedotin-piiq	**Polivy**	**Humanized**	**Diffuse large B-cell lymphoma**	**2019**	**[166]**
Brolucizumab	**BEOVU**	**Humanized**	**Macular degeneration**	**2019**	**[167]**
Teprotumumab-trbw	**Tepezza**	**Fully Human**	**Thyroid eye disease**	**2020**	**[168]**
Satralizumab	**Enspryng**	**Humanized**	**Neuromyelitis Optica spectrum disorder**	**2020**	**[148]**

Inebilizumab	**Uplizna**	**Humanized**	**Neuromyelitis Optica spectrum disorder**	**2020**	**[188]**
RA - rheumatoid arthritis; HIV - human immunodeficiency virus					

Risankizumab

Risankizumab (brand name SKYRIZI) is a humanized IgG1 mAb that was developed in partnership between AbbVie and Boehringer Ingelheim, for the treatment of immunological and inflammatory disorders. This biopharmaceutical binds with high affinity to and neutralizes the p19 subunit of IL-23, thereby inhibiting the proinflammatory effects of IL-23 [165,167]. IL-23 regulates the inflammation in the peripheral tissues, especially in type 1-polarized T-cell-driven disease. *In vitro*, risankizumab was able to inhibit IL-23-dependent phosphorylation of STAT3 in human B-lymphoblastoid cell lines originated from human diffuse large cell lymphoma, whereas *in vivo* (in mouse splenocytes) it inhibited the induction of IL-17 production from human IL-23 stimulation [165]. The recommended dosage of this biopharmaceutical is 150 mg (two 75 mg subcutaneous injections), but it carried out some risks of infections, including the activation of tuberculosis sepsis, cellulitis, and pneumonia. In the phase I of clinical trials, patients received a single dose of this mAb, where some of them received via intravenous, subcutaneous and a control group received the placebo (solution of 0.9% NaCl). At the end of week 12, 87%, 58%, and 16% of the patients achieved a decrease of 75%, 90%, and 100% in the Psoriasis Area and Severity Index (PASI), regardless of the dose [189,190]. In phase II, several patients received subcutaneous injections of risankizumab or ustekinumab and also the placebo. At week 12, 77% and 40% of the patients achieved a decrease of 90% or higher in the PASI, for risankizumab or ustekinumab, respectively [190]. To evaluate the efficacy, safety and tolerability, a third phase program, with four random clinical trials (IMMvent (NCT02694523), IMMhance (NCT02672852), ultIMMA-1 (NCT02684370), and ultIMMa-2 (NCT02684357)) was conducted in Asia, Canada, Europe, Mexico, South America, and the United States, comparing risankizumab to ustekinumab, adalimumab and placebo in the indication of plaque psoriasis [165,167,189]. In the end of these 4 trials, risankizumab showed more efficacy than placebo and more tolerability. On 26th March 2019, it was first approved in Japan for the treatment of plaque psoriasis, generalized pustular psoriasis, erythrodermic psoriasis and psoriatic arthritis, and on 23rd April 2019, FDA approved the treatment of moderate to severe plaque psoriasis. By the end of April 2019, this mAb had been granted approvals in Canada, US, and EU. Nevertheless, there are still many clinical trials ongoing, namely in Brazil and Russia, for the treatment of psoriatic arthritis, Crohn's disease, ulcerative colitis and atopic dermatitis [165,167,189].

Polatuzumab vedotin-piiq

Polatuzumab vedotin-piiq (brand name Polivy) is a humanized IgG1 conjugated to the antimitotic agent monomethyl auristatin E (MMAE) that was developed by Genentech and Roche as an antibody-drug conjugate designed for the treatment of hematological malignancies. Its target is CD79b, which is a B-cell receptor component, moderately to strongly expressed in lymphoma covalently conjugated via a cleavable linker to the MMAE. After the internalization and linker cleavation of Polivy, the released MMAE inhibits cell division and tempts apoptosis [166,167]. Polatuzumab vedotin-piiq displayed activity against most diffuse large B-cell lymphoma cell lines evaluated *in vitro*, regardless of whether they were of the activated B-cell-like (ABC) or germinal center-like cell-of-origin subtype or harbored mutations in CD79B known to be associated with poor survival in diffuse large B-cell lymphoma. *In vivo* (mouse xenograft models), Polatuzumab vedotin-piiq enhanced the apoptosis and reduced the proliferation of mature CD79b+ B-cell NHL cell lines, increasing the overall survival [166]. Its recommended dosage is an intravenous infusion of 1.8 $mg \cdot kg^{-1}$ in combination with bendamustine plus rituximab, but included common side effects - cytopenia [166,167,191,192]. There were several clinical trials to test the use of Polatuzumab vedotin-piiq in combination with immunotherapy, immunomodulating therapy, chemotherapy and as monotherapy. However, its approval by the FDA was based on evidence from one study GO29365 (NCT02257567), an open-label, multicenter clinical trial that included a cohort of patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL), that was steered in the US, Canada, Europe, and Asia [166,167]. The phase Ib safety run-in included 6 Polivy combined with bendamustine and Rituximab (P+BR) –treated patients, where the best response by the independent review committee was a complete response (CR) rate of 50.0% and an overall response of 50.0% [166,191,192]. In a phase Ib/II expansion it was evaluated Polivy plus bendamustine and Obinutuzumab (P+BG)-treated patients, and the best results by the independent review committee was a CR rate of 37.0%, an overall response of 48.1% and an average survival of 10.8 months [166,167,191,192]. In phase II, patients were randomized to receive an intravenous therapy of Polivy (1.8 $mg \cdot kg^{-1}$) with bendamustine (90 $mg \cdot m^{-2}$) and rituximab (375 $mg \cdot m^{-2}$) (P+BR) or BR alone, during 21 days for 6 cycles. Efficacy was founded on CR rate and duration of response (DOR), established as the time the disease stays in remission. At the end of the treatment, by PET/CT (Positron Emission Tomography – Computed Tomography) scans, the best responses by the independent review committee was a CR rate of 50.0% with P+BR and 22.5% with BR alone, an overall response of 62.5% with P+BR compared with 25.0% with BR and a median overall survival of 12.4 and 4.7 months, respectively [166,167,191,192]. In 10th June 2019, Polatuzumab vedotin-piiq was approved in the US by FDA in combination with the chemotherapy bendamustine and a Rituximab product for the treatment of relapsed/refractory diffuse large B-cell lymphoma – a rare type of white blood cells cancer. In the end of January 2020 it was approved for medical use in EU, by EMA [166,167]. Despite this approval there are still numerous clinical trials enduring, for instance a phase Ib/II with Polivy in combination with an immunomodulating agent, a phase III comparing Polatuzumab vedotin-piiq plus rituximab-CHP with rituximab-CHOP and a phase Ib/II trials which are evaluating Polatuzumab vedotin-piiq in combination with other immunochemotherapy [166].

Brolucizumab

Brolucizumab (brand name BEOVU) consists in a humanized single-chain antibody fragment (scFv) that was industrialized by ESBATech, Alcon Laboratories, and Novartis for the treatment of exudative (wet) age-related macular degeneration (AMD), diabetic macular edema and macular edema secondary to retinal vein occlusion [193]. This biopharmaceutical binds to the 3 major isoforms of human VEGF-A (VEGF110, VEGF121, and VEGF165), thereby interfering with their interaction with receptors VEGFR-1 and VEGFR-2, leading to the suppression of endothelial cell proliferation, neovascularization, and vascular permeability. By blocking VEGF-A, Brolucizumab reduces the blood vessels growth and controls the leakage and swelling [167,193]. *In vitro* BEOVU achieved a Ka of 28.4 pmol/L and an IC_{50} of 0.86 for the biding between VEGF165 and VEGFR2, which induced proliferation of human umbilical vein endothelial cells. *In vivo* (cynomolgus monkeys), after one intravitreal injection parallel clearance from all ocular compartments was observed [193]. The recommended dosage regime is 6 mg (0.05 ml of a 120 $mg \cdot ml^{-1}$ solution) via intravitreal injection monthly, despite that hoard side effects, as blurred vision, cataract, conjunctival hemorrhage, increased intraocular pressure, among others [193]. In a SEE study: phase I/II, a single dose of an intravitreal injection of Brolucizumab (0.5, 3, 4.5, or 6 mg) was compared with Ranibizumab 0.5 mg, which consisted in dose-escalation phase of Brolucizumab to the maximum feasible dose [167,193,194]. In the OWL study: phase II (NCT01849692) brolucizumab was compared once again with Ranibizumab, using microvolume injections (1.2 $mg \cdot 10\ \mu L^{-1}$ and 0.6 $mg \cdot 10\ \mu L^{-1}$) and infusions (1.0 $mg \cdot 8.3\ \mu L^{-1}$ and 0.5 $mg \cdot 8.3\ \mu L^{-1}$) and both stages demonstrated an effective comeback to BEOVU injection, with 70% and 80% rates in stages 1 and 2, respectively, and a rate of 60% in the brolucizumab infusion [167,193,194]. In the OSPREY study: phase II (NCT01796964), several patients were randomized to compare the intravitreal injection between Brolucizumab (6 $mg \cdot 50\ \mu L^{-1}$) and aflibercept (2 $mg \cdot 50\ \mu L^{-1}$). The treatment regime encompassed 3 treatment periods, week 8, week 32 and week 44 for Brolucizumab, with the aflibercept group maintained on q8-week dosing. At week 40 (q12-week), this study demonstrated that a 61% of Brolucizumab-treated eyes had a non-appearance of intraretinal fluid and subretinal fluid and these results gave crucial information for the study design and end points of the Phase III studies [167,193,194]. The optimal treatment and dosing regimen for patients (q8 weeks or q12 weeks) were determined by HAWK and HARRIER Studies: Phase III. In HAWK (NCT02307682) and HARRIER (NCT02434328) studies, Brolucizumab and aflibercept were administered through intravitreal injection at weeks 0, 4, and 8, then once every 12 weeks unless disease activity was exhibited. Brolucizumab successfully finalized phase III, demonstrating its efficacy and safety, while reducing treatment burden associated with regular IVT injections by achieving a result of 55.6% and 51.0%, maintained in the dose range of q-12 week afterwards the loading phase until week 48 in HAWK and HARRIER, respectively [167,193,194]. On 9th October 2019 it received its first approval by FDA for the treatment of wet AMD. BEOVU was the first anti-VEGF approved that offered greater fluid resolution versus aflibercept and also the ability to maintain eligible wet AMD patients on a three-month dosing interval immediately after a three-month loading phase. By the end of

February 2020 its use was approved in the European Union [167,193,194]. Several phase III studies comparing brolucizumab and aflibercept in patients with diabetic macular edema and retinal vein occlusion are undergoing [193].

Teprotumumab-trbw

Teprotumumab-trbw (brand name Tepezza) is a fully human IgG1 developed by the Horizon Therapeutics and used for the treatment of thyroid eye disease. This antibody binds to insulin-like growth factor-I receptor (IGF-IR) and blocks its activation and signaling; however, the exact mechanism of its actuation as a drug has not been fully strongminded and no official studies have been conducted [168]. The recommended prescription is an initial dose of 10 $mg \cdot kg^{-1}$ intravenous infusion and then a dose of 20 $mg \cdot kg^{-1}$ every three weeks for 7 additional infusions. Similarly to any other drug, it has some risks associated, like muscle spasms, nausea, alopecia, diarrhea and fatigue [168]. In 2016, a phase I trial in patients with diabetic macular edema was completed [167,168,195]. Tepezza was approved based on the results of two clinical trials, Trial I/ NCT01868997 and Trial II/ NCT03298867. The phase II trial, was a multicenter, double-mask and placebo-controlled study, where Teprotumumab-trbw was administered intravenously to patients (10 $mg \cdot kg^{-1}$ for the first infusion and 20 $mg \cdot kg^{-1}$ thereafter) and placebo (8 infusions), during 3 weeks. At week 24, it showed a greater than two-millimeter reduction in proptosis (eye protrusion) in 71.4% of the teprotumumab-treated patients, as compared with 20% of the placebo-treated ones [167,168,195]. In the phase III trial, patients with active thyroid eye disease were randomized the same way they were in phase II. At week 24 (primary outcome) a reduction in proptosis was observed in 83.0% of Teprotumumab patients versus 10.0% of placebo patients. Moreover, the results from orbital imaging, made in patients treated with Teprotumumab, revealed reductions of the orbital fat volume and in the extraocular muscle [167,168,195]. On 21st January 2020, Teprotumumab received its first approval in US by FDA for the treatment of thyroid eye disease. A specific clinical trial is currently ongoing, consisting in an extension of the phase III OPTIC trial, OPTIC-X (NCT03461211), with the purpose to provide access of this biopharmaceutical for patients with thyroid eye disease when no satisfactory alternative therapy is available [168].

Satralizumab

Satralizumab (brand name Enspryng) is a humanized immunoglobulin G2, which production resorts to Chinese hamster ovary cells and using recombinant DNA technology. It was developed by Chugai Pharmaceutical and Roche for the treatment of neuromyelitis optica spectrum disorder (NMOSD) [144,163]. It is a very recent mAb and the exact mechanism of its action is still unkown. Although, it is believed that it binds to the IL-6 receptor, blocking IL-6 signaling paths, reducing inflammation and IL-6 mediated autoimmune T- and B-cell activation, preventing differentiation of B cells into anti-aquaporin-4-IgG secreting plasma blasts [148,167]. *In vitro*, Enspryng allowed a reduction of NMO-induced BBB dysfunction. *In vivo*, subcutaneously administered Enspryng, inhibited in a significant way the IL-6 receptor signalling for four weeks, with increases in soluble IL-6 receptor levels observed in Japanese and Caucasian healthy volunteers and patients with rheumatoid arthritis or with

NMOSD [148,167]. A dosage of 120 mg is recommended at week 0, 2 and 4 as loading doses, followed by a maintenance dose of 120 mg every 4 weeks [148]. In phase I of the clinical trial, two studies were performed. SA-001JP was a single dose study in healthy volunteers, where the doses ranged from 30 to 240 mg [196]. SA-105JP was a multiple dose study in patients with rheumatoid arthritis, where they received a loading dose of 120 mg of Satralizumab at weeks 0, 2, and 4, followed by three further doses of either 120 mg, 60 mg, or 30 mg, also at four week intervals [196]. Within the first 28 days of SA-001JP, serum C-reactive protein levels were underneath the limit in all participants who received 120 mg and 240 mg, suggesting the efficient IL-6R blocking. In SA-105JP, the results showed that a loading dose of 120 mg at Weeks 0, 2 and 4, followed by 120 mg, resulted in a stable IL-6R concentration for the duration of treatment in patients with rheumatoid arthritis [196]. Enspryng's approval was based on robust data from a two phase III of clinical trials, SAkuraStar (NCT02073279) and SAkuraSky (NCT02028884), in patients with anti-aquaporin-4-IgG seropositive and seronegative [148,167,196]. Patients were randomized to receive Satralizumab subcutaneously (120 mg) or saline placebo at weeks 0, 2, 4, and every 4 weeks thereafter for a maximum duration of 1-5 years [148,167,196]. The monotherapy study, SAkuraStar, demonstrated that Satralizumab considerably reduced the risk of relapse versus placebo by 55% in all representative NMOSD's patients [148,149,167,196]. The baseline immunosuppressant therapy, SAkuraSky, included azathioprine, mycophenolate mofetil or oral corticosteroids at stable doses. Satralizumab lowered the risk of relapse versus placebo by 62% in NMOSD's patients, including anti-aquaporin-4-IgG positive and negative patients. The proportion of relapse free at weeks 48 and 96 was 89% and 78% with Satralizumab and 66% and 59% with placebo, respectively. The threat of relapse in patients who received Satralizumab added to immunosuppressant treatment was lower in comparison with those that received the placebo [148,149,167,196,197]. Based on the two phase III trials, Satralizumab showed to have a favorable safety profile and to be generally well tolerated when administered as a monotherapy or as an add-on therapy to baseline immunosuppressant therapy in patients with NMOSD [148,149]. Despite all promising results, it should be remarked that Satralizumab reported adverse effects, including headache, arthralgia and injection related reactions [148]. On 17th August 2020, Satralizumab was approved in the US by FDA for the treatment of NMOSD in adult patients who are anti-aquaporin-4-IgG positive. The open-label extension periods of phase III SAkuraStar (NCT02073279) and SAkuraSky (NCT02028884) trials are currently ongoing [148,167].

Given the aforementioned information, during 2020 many mAbs have been approved for the treatment of a wide range of diseases, especially inflammatory ones. In addition to the approved mAbs, there are still many FDA and EMA clinical studies underway, for instance Narsoplimab (Omeros Corporation), Tanezumab (Pfizer, Eli Lilly and Company), Etrolizumab (Roche) and Netakimab (BIOCAD) [167].

CONCLUDING REMARKS

Antibodies are proteins produced by vertebrates to help the immune system to fight viruses, bacteria, or other pathogens by recognizing a specific antigen. Due to advances on biotechnology and biomedicine it became possible to produce mAbs in high titers using mammalian cell technology. The development of new technologies and all characteristics associated to mAbs (specificity, selectivity and affinity) turn them important diagnostic tools and "magic bullets" for therapeutic purposes, covering a wide range of diseases, such as inflammatory disorders.

Inflammation is part of the body's natural immune response; however, an excessive response can last for months and years, causing tissue damage and leading to undesirable pathological manifestations such as cancer, diabetes, and cardiovascular, neurological, and chronic inflammatory diseases. Therefore, it is important to count with bioproducts able to decrease the inflammatory process, eliminating its potentially toxic products, and that can be used to treat long-term inflammation without (or with reduced) side effects, such as mAbs biopharmaceuticals.

In the current chapter, it is provided a global vision on the state-of-the-art concerning the characteristics, features and possible applications of mAbs, in particular for inflammatory diseases. The main molecular mechanisms and molecules involved in the inflammatory process were overviewed, while presenting some new mAbs-based treatments. There are over 150 mAbs currently being evaluated in clinical trials or as candidates for approval by the FDA and EMA, and over 85 mAbs are already approved by FDA (and/or EMA). Several studies demonstrated mAb-based therapies approved by FDA for the treatment of inflammatory diseases, such as Sarilumab and Brodalumab for rheumatoid arthritis and plaque psoriasis, respectively. More recent mAbs in this field comprise Risankizumab (plaque psoriasis), Polatuzumab vedotin-piiq (diffuse large B-cell lymphoma), Brolucizumab (macular degeneration), Teprotumumab-trbw (thyroid eye disease) and Satralizumab (neuromyelitis optica spectrum disorder).

Anti-IL6 and anti-IL6R mAbs were already approved by FDA, with the example of Tocilizumab, Siltuximab and Sarilumab showing high potential to fight inflammation. Tocilizumab has been particularly highlighted in the last months by the scientific and medical community due to its possible application for the treatment of severe patients with COVID-19.

All the works discussed in this chapter allowed to understand that the mAbs world is constantly increasing and gaining visibility, and there are several new/recently approved mAbs showing very promising results for the treatment of inflammatory diseases. This information should encourage both the testing and clinical trials of already existing mAbs that can have a positive effect in the treatment of several diseases, but also the search on new and more efficient alternatives to boost the widespread use of mAbs as conventional therapies for a wide range of diseases.

CONFLICT OF INTEREST

The authors (editor) declare no conflict of interest, financial or otherwise.

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