MONOCLONAL ANTIBODIES AND IMMUNOTHERAPY:

THEORY and APPLICATIONS

REVISION and UPDATE 2024

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FOREWORD

This text is an update of the previous document posted on the Papyrus site in June 2023. This update, in an educational spirit, takes into account recent publications listed on PubMed, monographs from the Wikipedia site but also and mainly it presents the monoclonal antibodies approved by the FDA in 2023 as well as their therapeutic targets.

If the first part devoted to the theoretical aspects of the immune reaction remains essentially the same in its presentation and its general references, we have tried to supplement these notions with recent data, relevant for the understanding of the different aspects of active and passive immunotherapy but also in the perspective of new applications.

Regarding active immunotherapy, we took into account the central role of messenger RNA in vaccination against the SARS-CoV-2 .

Finally, for passive immunotherapy using monoclonal antibodies we have described the new molecules recently approved by the FDA for therapeutic targets already mentioned in the previous publication but also for new targets. We kept the same presentation plan focused on the antigen targets. However, we have added recent references likely to help the reader in their quest for knowledge.

PART I
The fundamental immunology: A summary of current knowledge

INTRODUCTION

The word immunity originates from the Latin *immunis*: free of or preserved from. Immunity thus brings together all the innate and acquired mechanisms that the body has developed to protect itself against the biological aggressions of the surrounding environment and in particular those of the infectious agents.

These different but interrelated protective mechanisms are of three types. These are in order of increasing specificity:

1. Natural barriers: skin and mucous membranes.
2. The so-called non-specific innate immunity takes over if these first ones are overworked or injured.
3. Finally, acquired and specific immunity comes into play.

These three levels of protection are not isolated. On the contrary, they interact through cellular and soluble mediators, mainly cytokines.

In this first part we will summarize the current knowledge relating to these three levels of defense relevant to the understanding of the therapeutic effectiveness of immunotherapy and in particular that of monoclonal antibodies.

This therapeutic effectiveness is mainly based on the specificity of the reaction of an antibody with the antigen against which it is directed.

To this end, we will describe the molecular targets of therapeutic interest, underlying the antigen-antibody reaction. These are:

- The soluble mediators of the innate and the acquired immune reactions.
- The membrane structures and receptors specific to each cell type and responsible for the specificity of immunotherapy.
- Immunoglobulins, central mediators responsible for the specificity of immunotherapy.

We have indicated in blue italics the soluble mediators and cellular structures currently considered as targets for a monoclonal antibody for therapeutic purposes

I. The physical barriers of the body

The cells of the skin and mucous membranes with the chemical constituents of their secretions form the first non-specific line of defense of the body against the aggressions of the environment.

- Keratinocytes in the epiderm are not just a physical defense barrier. Thanks to the bactericidal power of the fatty acids and the lactic acid of perspiration, they also exert a protection of chemical type. These same keratinocytes possess receptors, which once activated by a microorganism, control the synthesis and the secretion of bactericidal substances and different cytokines.
- The mucous membranes of the internal surfaces are another potential gateway for intestinal, pulmonary or genitourinary pathogens. At these levels, mucus secretion participates in the protective mechanism by blocking the adhesion of bacteria to the epithelial surface.
- At the intestinal level, one should not underestimate the antagonism between different microbial species of the microbiota through different products of their metabolism.
- Finally, let us mention the bactericidal power of milk lactoperoxidase or the lysozyme of tears, a powerful neuraminidase that hydrolyzes microbial peptidoglycans.

- **A reminder: *Cytokines***

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- Cytokines are soluble messengers, autocrine or paracrine, of polypeptide nature (15-25 kDa) secreted by cells as different as macrophages, Natural killer, endothelial and epithelial cells, lymphocytes or fibroblasts.
- They are active at very low concentration (nM, pM) and the specificity of their activity depends on the pathophysiological context and the nature of the cells involved both in their synthesis and in their activity.
- Among the cytokines, there are *interleukins* and *chemokines*.Specific receptors are responsible for their activity.
- Interleukins provide communication between leukocytes. Interleukins act between two cells of the innate or specific immune response or both. About forty interleukins have been described today.
- Chemokines are cytokines with chemotactic properties. Peptides of low molecular weight (8-10kDa), they ensure that the right cells are in the right place at the right time.
- Chemokines are subdivided into two groups, homeostatic and inflammatory. These guide the phagocytic cells to the inflammatory site. Thus, among their different properties, chemokines, have the ability to attract and guide other cells, phagocytes and lymphocytes, to the inflammatory focus to activate and induce their differentiation. Among other things, they increase the specific immune response that reinforces the innate response.
- In addition, chemokines can act as agonists or antagonists by stimulating one or more receptors on more than one cell type.
- Other cytokines are growth factors (CSF: colony stimulating factor), have cytotoxic activity (*TNFa*) or interfere with viral replication (*interferons*).
- Cytokins and particularly interleukins constitute an important group of specific targets for monoclonal antibody immunotherapy.

II. The nonspecific immune reaction

The nonspecific immune reaction formerly known as the local inflammatory reaction is rapid, even immediate. It occurs when the aggression agent has passed one of the natural barriers following an injury for example.

Currently it is accepted that this innate reaction is lacking in memory, it remains unchanged over time and does not improve with repeated contact with the same pathogen, contrary to the specific immune response.

To fulfill its mission, the nonspecific immune reaction has plasma factors and white blood cells of the granulocyte and monocyte lineage, among others.

Its main role is in the detection, identification and eventual destruction of molecular structures foreign to our body. Most of the time, they are bacteria or viruses. More rarely they are subcellular structures, such as

mitochondrial DNA, not directly accessible but released during cell destruction.

1. Plasma factors

1.1. Complement factors

1.1.1. Definition

The complement system, as defined by the Belgian immunologist Jules Bordet, is a set of more than twenty proteins, numbered according to the chronology of their discovery, activated into a cascade amplifier mechanism.

Upon activation, these complement factors are hydrolyzed into two or more fragments, the main one being endowed with protease activity, activating the next factor in this cascade.

1.1.2. Activation pathways

Three known paths lead to the activation of the C3 factor which occupies a central place in this system:

- The factor C1 composed of C1q, *C1s* and C1r is activated by immunoglobulins G or M constituting antigen-antibody (Ag-Ab) complexes and trigger the activation of the classical pathway.
- The alternative pathway is activated by microbial wall polysaccharides and endotoxin.
- Finally, other molecules such as C-reactive protein (CRP) and some lectins whose collectins able to bind microbial polysaccharides can also activate the classical complement pathway.

1.1.3. Complement factors of physiopathological interest

Upon activation, these complement factors are hydrolyzed into two or more fragments, the main one being endowed with protease activity, activating the next factor in this cascade.

These 3 activation pathways, via the C3 factor, a central part of the complement system, leads to a common effector pathway that generates different protein fragments endowed with a physiological activity mediated by cell receptors. The products of this common core activation cascade contribute to the protective mechanisms of innate and specific immune reactions. We mainly mention:

a. Factors C3a, C4a and *C5a* are proinflammatory peptides by their activation of neutrophils, endothelial cells, but especially mast cells.

They are anaphylatoxins, they stimulate the release of various proinflammatory mediators including histamine from basophilic polynuclear cells and mast cells.

b. Factor C3b is mainly an opsonin. Its formation during activation of the alternate pathway in contact with a bacterial wall leads to its binding to the microbial agent by a coating phenomenon called opsonisation which facilitates its phagocytosis. In other words, it sets the table for those who will eat, phagocytes (φαγειν).

c. The MAC (membrane attack complex) formed by C5b-C9 factors increases the cell membrane permeability by perforating it, it is responsible for cell lysis and apoptosis.

Different cellular and plasma inhibitors regulate the activation and activity of complement factors. Among these, let us quote the best known, the C1 esterase inhibitor which, as its name suggests, inhibits the protease activity of the C1 factor but also that of *kallikrein*. Its genetic deficit, quantitative or qualitative, is responsible for angioedema. An anti-kallikrein monoclonal antibody has been developed for the replacement treatment of this quantitative or qualitative genetic deficiency. As we will see later, two membrane proteins, respectively *CD55* and *CD59,* play an important role in the control of C5a activity.

1.2. The proteins of the acute phase response (APP) of inflammation

APP are synthesized in the liver under the effect of interleukins (IL) and in particular *IL-1* released at the inflammatory site. They contribute to the regulation of this site and their plasma concentrations reflect its level of activity.

Among these proteins, we must mention CRP, a pentraxin, binds to phopholipids of the microbial wall in the presence of calcium (Ca^{++}), activates the classical pathway of complement with the consequence, among others, the formation of anaphylatoxins.

Among the other plasma factors responsible for the regulation of the local inflammatory site are the proteins of the coagulation cascades and the kallikrein-kininogen-kinin system.

2. Phagocytic cells

Phagocytic cells belong to the myelocyte lineage that includes polynuclear cells or granulocytes, monocytes and macrophages, but also mast cells and dendritic cells.

Blood polymorphonuclear leucocytes and tissue macrophages, both grouped under the heading of phagocytes, are mainly responsible for phagocytosis, the center of the non-specific immune reaction.

2.1. Polymorphonuclear leucocytes

Among polynuclear leucocytes, neutrophils are the most powerful, they are able to destroy bacteria and extracellular yeasts while parasites (helminths) are the preferred prey of eosinophils and basophils.

Following vasodilatation caused by kinins, an increase in vascular permeability due to cytokines but also to histamine released from mast cells and the production of anaphylatoxins (mainly C5a and C3a), neutrophils will migrate to the inflammatory site by diapedesis and chemotaxis on the influence of IL-8 and chemokines**.**

This diapedesis involves an interaction between endothelial integrins and leukocyte adhesion molecules. This diapedesis is facilitated by an increase of endothelial cell adhesiveness induced by different interleukins among which *IL-1β and TNF-α.*

At the inflammatory site, polymorphonuclear neutrophils enhance the phagocytic activity of macrophages.

Then comes a wave of recruitment of blood monocytes by a similar mechanism if not identical to that of neutrophils.

2.2. Macrophages

Unlike polymorphonuclear cells, which have a predominantly blood localization, macrophages have a tissue localization, even if some derive from blood monocytes. They are present in the basement membrane of the blood vessels and in the connective tissue where they are known by different names, for example, hepatic kupffer cells, mesangial kidney cells or bone osteoblasts, among others.

2.3.Dendritic cells

2.3.1-Definition

a. Dendritic cells are mononuclear cells, they also originate from bone marrow, they are close to macrophages with which they share a main function: phagocytosis.

   Their name, dendritic cells, comes from a particular morphological characteristic, the presence of dendrites or membrane projections which allow them close contact with the surrounding environment.

b. Immature dendritic cells are ubiquitous, particularly present in epithelia, the entry point for infectious agents where they play the role of sentinels. In the skin, for example, they are called Langerhans cells.

c. Dendritic cells capture infectious agents, neoplastic cells and prepare their antigens (Ag) to present them to T lymphocytes after having migrated into the lymph nodes, hence their name antigen presenting cells (APC).

   The presentation of Ag to T lymphocytes is a pivotal step in the immune reaction; a quantitative or qualitative abnormality is associated with an autoimmune or immunodeficient disease.

   Dendritic cells therefore play an essential role in the relationship between the innate immune reaction and the specific immune reaction.

2.3.2. Which cells are likely to present an antigen?

In the broad sense, cells equipped with major histocompatibility complex (MHC) type I, in theory all cells except red blood cells, can present an antigen. In a more restricted and specific sense, APC originating from dendritic cells provided not only with MHC type I but also with MHC type II are the cells preparing the Ag at the level of the non-specific immune reaction to present it to the specific immune reaction effector cells, T lymphocytes.

We will see that B lymphocytes are also equipped with such presenting capacities but this time at the level of the specific immune reaction.

For educational purposes, we will focus the rest of our presentation on APC originating from dendritic cells.

2.3.3. What are the different stages in the preparation of an antigen by an APC for its presentation to T lymphocytes?

The preparation of an antigen for presentation to T lymphocytes is a complex phenomenon which includes several stages: recognition, capture, metabolism and finally presentation of the antigen to T lymphocytes in a form that they can recognize, associated with a MHC molecule. Indeed, unlike B lymphocytes, T lymphocytes cannot recognize an antigen in its native form, but rather short peptide sequences derived from this antigen.

a. Recognition of a pathogen

After crossing a natural barrier in the body, a blood Ag is taken over by the spleen. If it has penetrated a tissue, it is transported in the lymph by a dentritic cell to a lymph node.

This support is carried out by membrane receptors, the PRR (pattern recognition receptors) which identify the chemical structure of the intruder, of the non-self.

Thanks to these receptors, dentritic cells can recognize structures, microbial or viral molecules known as PAMP (pathogen-associated molecular patterns). They can also detect DAMP (damage-associated molecular patterns) resulting from tissue damage or a neoplastic phenomenon. These receptors therefore provide information as the nature of the foreign structure, of the non-self, on the one hand and its extra or intracellular location, on the other hand.

A number of these receptors have been identified, including endocytosis receptors and signaling receptors. These receptors have been characterized and classified. Signaling receptors include TLR (toll-like receptors).

b. Cellular activation

The binding of a ligand to one but preferably to several PRR molecules facilitated by its prior opsonization by the C3b factor triggers a cascade of intracellular signals. These cellular activation phenomena can be summarized as follows:

- Formation of pseudopods and engulfment of the intruder in a vacuole (phagosome for an extracellular protein, endosome for an intracellular protein).
- Discharge of lysosomal hydrolases into these phagosomes. These participate in the destruction of the intruder by the reaction of oxygen and nitrogen radicals formed in the oxidative respiratory burst. In the case of an intracellular protein, it is metabolized after ubiquitination by the proteasome dependent on the Golgi system.
- Activation of a TLR triggers a transduction signal which, via a cascade of intracellular second messengers, induces the transcription factor NF-κB which leads to the synthesis of pro-inflammatory cytokines. These amplify the non-specific immune reaction. They modify vascular permeability, thus facilitating the diapedesis of blood cells and the exudate of plasma proteins. In addition, they prepare the specific immune reaction by stimulating the secretion of *IL-12*.

c. The presentation of the Ag

Once the preparation of the Ag is completed, this dendritic cell migrates towards a lymph node and undergo a transformation phenomenon which reduces its phagocytic function in favor of the presentation of the Ag to the T lymphocytes, becoming an APC, expressing MHC molecules and a number of receptors for chemokines.

These interdigitated dendritic cells that have become APC play a central role in coordination and contact with T lymphocytes. MHC-I molecules bind and present endogenous antigens from tumor cells, viruses or bacteria of intracellular location. MHC-II molecules, on the other hand, have as ligands peptides derived from extracellular or exogenous antigens metabolized by the endocytotic pathway.

This change in function, from dendritic cell to APC, is associated with the overexpression of 2 ligands B7.1 (CD80) and B7.2 (CD86) on the surface of APC, overexpression controlled by NF-κB.

Certain cytokines (*IL-1*, *TNF-α* or even GM-CSF: granulocyte-macrophage colony-stimulating factor) produced by macrophages and polymorphonuclear cells can also induce this change in function.

Useful references to learn more:

- Stagg, A.J., Hornsby, E. and Knight, S.C. In: John Wiley & Sons online library. Encyclopedia of Life Sciences (2020). doi:10.1002/9780470015902.a0029138.
- Gaudin J. and Kumar P. Front Immunol. March 06, 2019. Volume 10. doi: 10.3389/fimmu.2019.00360.
- Schuijs M.J.,Hammad H.and Lambrecht B.N. Trends in Immunology;2019, 22-44. doi:10.1016/j.it.2018.11.001.
- Cabeza-Cabrerizo M,, Cardoso A.et al. Annu Rev Immunol. 2021;39:131-166.doi:10.1146/annurev-immunol-061020-053707.

2.4. Natural Killer (NK) cells

2.4.1. Definition

a. Just like B and T lymphocytes, NK cells constitute a population, the third, of lymphocytic or lymphoid cells according to the authors. They derive from a common lymphoid precursor present in the bone marrow, the primary lymphocytic organ, but also in secondary lymphocytic organs such as the spleen and lymph nodes.

   Large in size, NK cells are present in the blood, the liver, particularly the spleen and associated mucous membranes. NK cells represent 5-15% of circulating mononuclear cells.

   Different subtypes of NK cells have been described based on their tissue localization and membrane markers. NK cells therefore do not constitute a homogeneous cell population.

b. Identified by Herberman in 1976, NK cells are not immunocompetent cells strictly speaking, they lack specificity and perhaps memory. However, like cytotoxic T lymphocytes, NK cells have cytotoxic power against tumor cells.

c. The presence of different membrane markers including CD56 (CD: cluster of differentiation) and CD16 allows them to be identified. Unlike T lymphocytes, NK cells lack CD3 and TCR (T cell receptor). In addition, the membrane of NK cells exhibits receptors that inhibit and activate their cytotoxic activity.

2.4.2. NK: cytotoxic cells

Within the innate immune system, NK cells play a key role in the cytotoxic response to bacteria, viruses and tumor cells. They also play a protective role in the development of autoimmune diseases including lupus erythematosus, for example.

This cytotoxic role is mediated by membrane receptors which detect cellular abnormalities induced by tumor development, infection or stress. Unlike T lymphocytes, the cytotoxic properties of NK cells do not depend on specific receptors but on constitutive receptors. The latter do not result from a gene reorganization as is the case for the specificity of the immune reaction.

NK cell membrane receptors are of two types: inhibitors and activators of cytotoxicity. Upon contact with a normal cell, the inhibitory cytotoxicity receptors take over the activating receptors.

2.4.3. Privileged targets of NK cells

The cytotoxicity activating receptors are stimulated by a change, an anomaly of the cell membrane of the target lacking all or part of the MHC molecules.

This anomaly signs a mutation induced during neoplastic transformation, following the presence of a foreign protein such as the hemagglutinin of the influenza virus, or any intracellular viral infection accompanied by the production of *IFNγ* which interferes with protein translation.

2.4.4. The cytotoxic mechanisms

To kill cells infected by a virus or in the process of neoplastic transformation, NK cells have 2 particularly effective weapons:

a. Death of the target cell by apoptosis. This is mediated by death receptors on the surface of the target cell. These receptors belong to the TNF receptor superfamily. If one of these receptors meets its ligand (*TNF-α*, Trail or Fas), this binding leads to activation of the caspase pathway and death of the target cell by apoptosis.
b. The second mechanism of cytotoxicity involves the formation of a synapse between the target cell and the NK cell. This synapse allows the NK cell to discharge the contents of its cytotoxic granules, perforin and proteases (granzyme) into the short space separating the two types of cells. This results in the death of the target cell by apoptosis after perforation of its membrane and proteolysis of cellular structures.

2.4.5. NK cells can also amplify the immune response by different mechanisms:

a. The production of *IFNγ*. This increases the phagocytic activity of macrophages accompanied by the synthesis and secretion of pro-inflammatory cytokines such as *IL-12* which shapes the T lymphocyte response and the presentation of Ag by APC.

   NK cells also release other cytokines, including IL-10 and GM-CSF, which recruit other cells to the inflammatory focus.

b. NK cells also have the capacity to present Ag associated with MHC class I to cytotoxic T lymphocytes.

c. NK cells possess an antibody-dependent cytotoxic system against Ag-Ac complexes, Ag being of soluble or cellular nature. The CD16a (FcγRIIIA) receptor for Fc fragments of Ab allows NK cells to kill their target by means of antibody dependent cell cytotoxicity (ADCC). We will come back to this in more detail.

2.5. A word about iNKT or NK1.1 cells

These cells share morphological and metabolic characteristics with both NK cells and T lymphocytes.

They weakly express the conventional T cell receptor. Unlike these, however, this receptor is of restricted specificity; it only recognizes lipid and glycolipid

molecules presented by the CD1d receptor, which is different from the membrane MHC molecules of APCs.

Useful references to learn more:

- Samg-Youg W, Fu T et al. Mol Cancer 19: article number120; 2020 (open access). doi :10.1186/s12943-020-01238 PMC7409673.
- Crinier A et al. Immunity 2018;20 : 971-986. doi: 10.1016/j.immuni.2018.09 .009. Ep
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- Souray P and Lalume G Front.Immunol.2017; 13:1124. doi.org/10.3389/fimmu. 2017.01124.

In conclusion

Thanks to its proteins, its cells, its soluble mediators, the innate immune response prepares the table for the so-called acquired specific immune response which fills these gaps.

Through their receptors, their cytotoxic mechanisms and the soluble mediators that they synthesize, APC and NK cells participate in the effectiveness of innate and specific immune reactions and therefore in the effectiveness of active and passive immunotherapy. We will illustrate this double participation with relevant examples.

The Major histocompatibility complex.

The major histocompatibility complexes (MHC) of class I and II are membrane glycoproteins, heterodimers with significant genetic polymorphism.

1. MHC class I is ubiquitous, it is expressed on the surface of many nucleated cells such as macrophages, dendritic cells, lymphocytes and cells of many organs. MHC class I is composed of a polypeptide chain of molecular weight (MW) equal to 44 kDa joined to a molecule of β2 microglobulin (MW: 12kDa).
2. MHC Class II is also a transmembrane glycoprotein composed of two chains: α (34kDa) and β(29kDa).
   Class II MHC expression is more restricted. In the basal state, only APC and B cells but not T cells, express MHC class II.
   Activation of these APC is responsible for its overexpression.
3. The MHC molecules of APC have the function of presenting a peptide to T lymphocytes. The MHC-peptide complex is formed before its expression on the surface of the APC. The length of this peptide, its formation from an endo or exocellular protein as well as the nature of the bonds involved in this peptide-MHC complex have been identified.
4. MHC Class I and Class II, synonymous with HLA (Human Leukocyte Antigen), are best known for their importance in organ transplantation and rejection. They also play an important role in the specific immune response. Indeed, the MHC class I and II molecules sound the alarm for the triggering of the specific immune reaction and the destruction of the infected cell either by apoptosis or by phagocytosis by two types of cytotoxic cells: NK cells and cytotoxic T lymphocytes.

III. The specific immune reaction

In contrast to the nonspecific, innate immune response, the specific, acquired or adaptive immune reaction is developed during repeated contact with the same pathogen, with the same antigenic structure. It focuses mainly on two types of cells of the lymphocyte lineage: T and B lymphocytes.

These cells are assisted in their tasks by other cells like NK cells, APC, as well as by the cytokines synthesized and secreted during the course of the nonspecific immune reaction.

Although specificity is the main or even essential characteristic of the acquired immune response, other features differentiate it from the innate immune response. For example:

- The chronology: about a week after the onset of the non-specific reaction. This time needed to synthesize the antibodies (Ab).
- It is flexible and adapted specifically to a pathogen and no longer to a class of coliform bacteria, for example.
- It has memory capacity (anamnestic response). It remembers a first contact and increases, improves with each new contact.

Specificity and anamnestic response underlie the principle of vaccination.

1. Lymphocytes and the immune system

T and B lymphocytes originate from the same stem cell in the bone marrow. T cell precursors migrate to the thymus where they differentiate into mature T lymphocytes. This process is accompanied by the synthesis of proteins on the surface of the cell membrane: receptors and membrane CD markers.

B lymphocytes develop in the bone marrow, in close contact with the glycoproteins of the non-lymphocytic cell stroma.

Thymus and bone marrow are the primary lymphoid organs from which lymphocytes migrate to secondary lymphoid organs such as lymph nodes, spleen, and lymphoid tissues associated with skin and mucous membranes. The lymphocytes circulate between these different tissues utilizing the blood and lymphatic circulations.

The antigens in free form or linked to dendritic cells are transported by the lymph in the subcapsular sinus of the lymphatic nodes in which there are 3 regions, cortical, paracortical and medullary, respectively, supported by a frame of stromal cells and collagen fibers. B and T lymphocytes are sequestered in two different regions. On the one hand, B lymphocytes are organized into cortical follicles, associated with follicular dendritic cells. On

the other hand, T lymphocytes are mainly found in the paracortex close to the medullary area.

2. T lymphocytes and the cellular immune reaction

T cells are responsible for the so-called cellular specific immunity. This one has the ability of clonal and memory cells proliferation as well as the humoral immunity for which B cells are responsible.

The nature of their CD makes it possible to classify T lymphocytes into two main groups: CD4+ T lymphocytes and CD8+ T lymphocytes. CD4+ T cells are the main lymphocytes in the circulating blood. T lymphocytes are different from B cells by the nature of their membrane receptors.

2.1. T Cell Membrane Receptors (TCR)

2.1.1. In contrast to B cells, T cell receptors are not immunoglobulins(Ig), although they have some degree of structural homology with them. Another difference also, these receptors are not present in plasma. The recombination mechanisms leading to their synthesis are similar to those which will be described for the synthesis of Ig of B lymphocytes.

2.1.2. These receptors are composed of 2 peptide chains joined by a disulfide bridge. The nature of these 2 peptide chains makes it possible to subdivide these receptors and therefore the corresponding T lymphocytes into γδ (TCR1) and αβ (TCR2). The latter are the majority while the first minority (1-5%) are mainly present in the epithelial lymphocytes.

The two chains γδ and αβ consist of a variable extra-membrane part and a constant transmembrane sequence. Likewise, the variable part comprises 3 hypervariable regions which bind a peptide complexed to a molecule of the membrane MHC of the APC.

2.1.3. The role of the TCR of CD4 + lymphocytes is to recognize and bind to a peptide presented by MHC class II on the surface of the APC. We have seen that this peptide comes from an extracellular protein previously metabolized by the lysosomal enzymes of this same APC.

The TCR of CD8+ lymphocytes have a similar function. However, they recognize only the peptides presented by the MHC class I. These peptides are of intracellular origin, resulting from the endosomal metabolism of a bacterial, viral or tumor protein.

2.2. T cell activation and cellular immune response

2.2.1. The T lymphocytes of the para cortex of the lymph nodes come into contact with the APC which present to them the Ag complexed with the MHC molecule. If the T lymphocyte recognizes this complex by its receptor (TCR), there is a binding. As we have seen, the antigen presented by the APC is a peptide derived from the intracellular metabolism of the native protein phagocytosed by this same dendritic cell. Unlike B lymphocytes, T lymphocytes can neither recognize nor react with a native protein.

2.2.2. The binding of the lymphocyte TCR receptor with the MHC molecule is of low affinity. It must therefore be consolidated by the formation of an immune synapse in which:

a. *CD3* and the protein ζ form the TCR complex. This complex consolidated by a co-receptor (CD4 or CD8) constitutes the center of this synapse. These co-receptors interact with the constant part of the MHC-peptide complexes on the surface of the APC.

b. Other proteins are added to the periphery of this complex, consolidating it and completing the formation of the immune synapse. Among these proteins are:

    - *Integrins*, present on T lymphocytes, bind to APC *adhesion molecules*.
    - The binding of the CD28 of T lymphocyte to the receptor B7.1 (CD-80) and B7.2 (CD-86) on the APC provides a second signal necessary for the activation of T lymphocytes.

These different bindings between APC and T cell results in a *supramolecular activation cluster* **(**SMAC) with the consequence of the clonal proliferation of T lymphocytes and their activation in effector cells.

2.2.3. This activation leads to a *polarization* phenomenon, in other words a differentiation of the T lymphocytes:

a. CD8 $^{+}$ lymphocytes give rise to cytotoxic T lymphocytes (Tc) which have the capacity to kill specifically, by a mechanism similar (perforin, granzyme) to that of NK cells, cells infected with a virus, bacteria or on the way neoplastic transformation.

    Cytotoxic CD8 $^{+}$ T lymphocytes also secrete *IFNγ* and *IL17*, contributing to the activation of phagocytes.

b. T helper (Th) lymphocytes are derived from CD4 $^{+}$ lymphocytes.

Under the influence of the signals described above but also that of the cytokines secreted by the cells of the innate reaction, these $CD4^+$ lymphocytes differenciate (polarization) into Th lymphocytes respectively:

- $CD4^+$ Th1: secrete *IFNγ* and control the activation of $CD8^+$ (Tc) cells and macrophages.
- $CD4^+$ Th2: secrete *IL-4, IL-5* and *IL-13* and activate the antibody response of B lymphocytes, in particular the synthesis of IgE.
- CD4 + Th17: secretes *IL-17* necessary for the activation of neutrophils.

c. Finally, regulatory T cells (Treg) are also $CD4^+$ lymphocytes ($CD4^+$ CD25). They control the immune response, preventing it from becoming aberrant, as is the case in autoimmune reactions.

2.3. Check points of cellular immune reaction

Two cellular proteins are of particular interest because they are the target of monoclonal antibodies used in oncology.

2.3.1. ***CTLA-4*** (cytotoxic T-lymphocyte antigen 4)

CTLA-4 is structurally related to CD28 of T cells and binds the B7.1 and B7.2 ligands (CD80 and CD86) of APC.

CTLA-4 is not detectable on resting T cells but is expressed within three to four hours after activation induced by TCR-CD28 complex. *CTLA-4* has a higher affinity for B7.1 and B7.2 and therefore competes with CD28 for binding with B7.

While the CD28-B7 complex is co-stimulatory, that CTLA-4-B7 helps to block the activation of TCR. Thus, *CTLA-4* moderates or prevents the stimulation of T lymphocytes.

2.3.2. ***PD-1*** (Programmed Death 1)

PD-1 is a potential inhibitor of T-cell receptors.

Like *CTLA-4*, *PD-1* belongs to the CD28 family of co-receptors. It mediates subsequent inhibitory effect by interfering in the intracellulary signaling pathway, after T lymphocyte activation.

PD-1 is activated by one of its two *PD-L1* or PD-L2 ligands, expressed on the surface of APC but also by tumor cells (*PD-L1*).

The therapeutic interest of check points lies in blocking the action of *CTLA4* and/or *PD-1* which reactivates the antitumor immune response.

3. The plasma immune reaction

3.1. B lymphocytes and the plasma immune reaction

3.1.1. B lymphocytes are responsible for the synthesis of antibodies (Ab), glycoproteins also called immunoglobulins (Ig), receptors for these B lymphocytes (BCR: B cell receptors). Unlike TCR, the synthetized Ig (BCR) at the surface of B cells are released into the bloodstream and extracellular fluids, once B cells differentiated into plasma cells

B2 lymphocytes or follicular B lymphocytes, present in the lymphoid follicles of the spleen and lymph nodes, are mainly involved in the so-called plasma immune response, thanks to their BCR which specifically recognize a single epitope of a protein antigen. B cells carrying such receptors are activated by this link. They undergo clonal proliferation into IgG (Ab) producing plasma cells and can also transform into memory cells.

3.1.2. These B lymphocytes, for their clonal proliferation and their transformation into memory cells, depend on cytokines and on Th lymphocytes and more particularly Th lymphocytes, present in the follicles of the lymph nodes (Thf).

Other B lymphocytes do not require the help of Thf lymphocytes to produce antibodies. Their BCR recognizes a linear Ag (endotoxin or microbial polysaccharides, for example) presenting the same epitope repeatedly which can bind simultaneously to several surface Ig. In fact, they provide wide protective coverage before specific B2 lymphocytes come into play.

3.2. Structure of Ig, membrane receptors of B lymphocytes (BCR)

3.2.1. The Ig molecule consists of two heavy chains (H, MW: 50kDa) and two light chains (L, MW: 25kDa) identical in pairs:

a. The nature of the L chains is either (*isotype*) κ or λ. The nature of the H chains, respectively α, γ, μ, d and ε, defines the major families (*isotypes*) of Ig: IgG, IgM, IgA, IgD and IgE.

b. *Haplotypes* represent the different genetic variants of the same isotype.

   The heavy chain of IgG makes it possible to identify four *isoforms* numbered from 1 to 4 (IgG1-IgG4), endowed with effector properties of variable strength.

c. Disulfide bridges between the L and H chains on the one hand, and between H and H on the other hand, ensure the stability of the molecular structure.

d. Finally, Ig can exist in different forms. This is the case of pentameric IgM whereas IgA is present in the form of dimers.

3.2.2. The chains V and H of the Ig consist of two parts. One variable (V) NH2 terminal and the other constant (C) COOH terminal. The variable part (VH and VL) of chains H and L corresponds to the last 100 amino acids of their N-terminal part. Their association (VH and VL) forms the *Fab* fragment capable of recognizing and binding the Ag. Thus, an Ig molecule has two Fab fragments.

3.2.3. These variable parts, respectively VH and VL, contain three hypervariable sequences, the complementary-determining regions (CDR1, 2 and 3), that form the binding site of the Ab for an epitope on the surface of an Ag. These CDR are responsible for the specificity of the binding of the Ab with its Ag. In this case we speak of the *idiotype* of the Ab.

3.2.4. The constant part, the terminal COOH of the two H chains forms the *Fc* fragment (c for crystallizable) responsible for the effector functions of Ig.

The effector functions of the different Ig vary according to their isotype:

a. Pentameric IgM and IgD are the B cell receptors for antigens.

b. Cellular receptors for Fc fragments of IgG, A and E have been characterized. They differ in their distribution and affinity. Special consideration must be given to the *FcRn* receptor that mediates the transport of maternal IgG to the fetus via the placenta.

c. Polymerized IgG as well as antigen-associated IgM can activate the complement. Under the same conditions, *IgE* activates mast cells and basophilic polynuclear cells, a phenomenon of degranulation leading to the anaphylactic reaction.

Ig G : archetype of Ig

Because of its pathophysiological role and its use as a reagent but also as a drug, the IgG molecule is the most studied.

Its structure comprises 12 homologous domains equipped with disulfide bridges. Two domains form the chain L (VL and CL) and four domains for the H chain. One VH and 3 CH (CH1-CH2-CH3) for the C-terminal part respectively.

The two parts V and C of the Ig are connected by a hinge region between the CH1 and CH2 domains. It is responsible, for example, for the flexible form of IgG, the spacing of the two Fab fragments can vary between 0^o (Y shape) and 180^o (T shape).

The CH2 domain of IgG opsonizing a particle is the binding site to C1q and to cellular receptors for the Fc fragment.

The Fc fragment therefore plays a role as important as the Fab fragment in the acquired immunity. Indeed, after reacting with an Ag (virus, bacterium, toxin, allergen, cytokine, receptor on a tumor cell...) via its Fab, the Ab subtracts the latter from the body by activating phagocytosis by its Fc fragment. By its Fc fragment also, the antibody is able to activate the antibody-dependent cell cytotoxicity (ADCC) of NK cells.

The cellular receptors for this Fc fragment are distinguished, on the one hand, by their different expression on the surface of phagocytic cells (polynuclear neutrophils, monocytes, macrophages, dendritic cells and NK) but also by their affinity for the four isoforms of the chain. γ (IgG1-IgG4). The difference in affinity for the Fc fragment of the γ chains makes it possible to distinguish 3 types (FcγR I-II-III) of cellular receptors. For example, FcγR1, expressed on the surface of macrophages and neutrophils, has a particularly high affinity for IgG1 and IgG3. The latter therefore play the role of opsonin activating phagocytosis The CH2 domain of IgG opsonizing a particle is the binding site to C1q and cellular receptors for the Fc fragment.

NK cells are also provided with receptors for Fc fragments of IgG (mainly CD16a, FcγRIIIA) allowing them to kill their target by ADCC (antibody dependent cellular cytotoxicity), followed by the release of cytokines including *IFN γ* .

As we will also see the structure of the Fc fragment and its effector functions play a vital role in the therapeutic effectiveness of monoclonal Ab, in particular those used in oncology.

3.3. Antibody synthesis

Each B lymphocyte can synthesize only one single Ab, of well-defined specificity (idiotype) in response to an epitope present on an antigen.

3.3.1. Once activated, one lymphocyte divides into all identical plasma cells forming a clone, it can also transform into memory cells. This is called clonal selection. T cells of the same specificity are also selected by an identical mechanism. The formation of a clone requires the cell division of one cell into thousands of others, all identical. This explains the latency required for the detection of circulating antibodies.

On the other hand, a second contact with the same antigen is accompanied by a faster and more important immune response for both B and T cells. It is the memory effect that underlies the notion of booster vaccination.

This clonal selection from a lymphocyte is important since blocked by antimitotic substances makes the patient more susceptible to infections.

3.3.2. The gene information necessary for the synthesis of L and H chains of an Ig is present in germ cells. However, this information is dispersed in the form of multiple gene segments on 3 different chromosomes: respectively chromosome 2 for the κ chain, chromosome 22 for the λ chain and chromosome 14 for the H chain.

Lymphopoiesis is accompanied by a somatic rearrangement (a recombination) of the different gene segments, this rearrangement is necessary for the formation of a final exon, the transcription into RNA and therefore to the synthesis of an Ab. This somatic recombination explains why the specific immune reaction is acquired and not innate.

It is the diversity of the combinatorial process between different gene segments that explains the diversity of the Ig repertoire. Additional diversity is achieved by combining the different H chains with the λ or κ chains.

3.4. Activation of follicular B lymphocytes and the plasma immune reaction

This reaction occurs in two stages and at two levels.

Indeed, as for the activation of T lymphocytes, two signals are required for the activation of B lymphocytes, thus limiting the phenomena of erroneous reaction including that directed against myself.

3.4.1. The first of these 2 signals is provided by the BCR co-receptor complex. Lymphocytes are stimulated on the surface of the so-called primary follicles. Dendritic cells present Ag to BCR of B cells. These cells are resident in the follicles of the lymph nodes.

-An epitope of this Ag, protein in the native state, reacts with the paratope of the membrane BCR, monoclonal IgM or IgD. Thus, unlike T cell receptors, these BCR can recognize an epitope of an antigen in their native state. By analogy with TCR, these BCR are associated with co-receptors; in this case Igα (CD79a) and Igβ (*CD79b*) responsible for the transmission of the intracellular transduction signal. The latter is necessary for the activation of the B lymphocyte.

This BCR complex is associated with other membrane proteins including CD21, a receptor for the C3d fragment with which it will be able to interact in the event of complement activation by immune complexes.

This Ag-BCR complex is internalized and metabolized into peptides in an endosome.

3.4.2-The second signal is triggered at the germinal center, the central part of the follicle, which is the site of significant metabolic activity.

a. A peptide, resulting from the proteolysis of the Ag-BCR complex, reappears on the surface of the B lymphocyte associated with an MHC class II molecule. The B lymphocyte therefore acts like a APC and presents the peptide-MHC complex to follicular Th lymphocyte (Thf). The latter derived from a CD4+T lymphocyte recognizes the peptide-MHC complex on the surface of the B lymphocyte if this peptide is identical to that presented by a APC in the activation phase of the cellular immune reaction. During this contact, there is a synapse formation and the binding of the MHC-B cell peptide complex to the TCR of the Thf lymphocyte.

b. This cellular contact leads to the binding of the B7 ligand of the B lymphocyte to the CD28 of the Thf lymphocyte and the overexpression of CD40L on the surface of the Thf lymphocyte allowing its binding with CD40 on the surface of the B lymphocyte, and thus completing the immune synapse. This is responsible for the proliferation of B lymphocytes and their differentiation into memory cells, plasmablasts and then long-lived medullary plasma cells. The latter, during repeated contact with the same antigen (eg vaccination) are the site of the change in isotype (isotype switching) and somatic hypermutations leading to the synthesis of antibodies with increased affinity (affinity maturation).

   Different cytokines (IL-21) also help B lymphocytes in their proliferation, the synthesis of high affinity antibodies, the differentiation and the class switching of the synthesized Ig. For example, the synthesis of IgG is induced by *IFN-γ* at least in mice, that of *IgE* is mediated by *IL-4* of Th2 lymphocytes, and the synthesis

of mucosal IgA is stimulated by transforming growth factor -ß (TGF-ß).

Finally, these different Ig molecules can be split from the membrane support of medullary plasma cells and released in soluble form in the plasma where they will participate in the antigen-antibody reaction.

In conclusion, the specific immune response is not an isolated phenomenon, but a complex outcome prepared by the innate immune reaction. In fact, the two immune reactions are in contact mainly by two types of innate reaction cells, NK cells and APC, which collaborate directly with T lymphocytes and indirectly with B lymphocytes. In addition, soluble mediators such as interleukins and chemokines ensure contact between these different cells and participate in their activation.

Two excellent references to learn more:

- The 13th edition (2017) of : Roitt's Essential Immunology publié chez Wiley Blackwell par P.J. Delves, S.J. Martin, D.R. Burton, I. M. Roitt.
- The ninth edition (2018) of : Cellular and Molecular Immunology publiè chez Elsevier par A.K. Abbas, A.H. Lichtman, S.Pillai.

PART II
The IMMUNE REACTION AND IMMUNOTHERAPY

I. The antigen-antibody reaction

1. The immunogen, the antigen, the hapten

1.1. Definition

The nature of the antibodies has already been defined, but more specifically that of therapeutically useful antibodies, namely immunoglobulin G (IgG).

Now what about the antigen that was used to induce the synthesis of this antibody?

Indeed, etymologically speaking, the antigen is the one that generates (gene) an antibody (anti-).

At present, this *antigen* function, that of generating an antibody, is replaced by the term *immunogen*. This seems logical since this antibody-generating function is only part of the specific immunological reaction for which the immunogen is responsible.

An antigen is therefore defined as a molecule able to react with a receptor of T and B lymphocytes, thus with an antibody in this last case.

The antigens are, let us recall, molecules , proteins, lipids, carbohydrates, bacteria, pollen : carriers of epitopes. These epitopes are three-dimensional structures recognized by the paratope of the Ab formed of the 3 hypervariable sequences (CDR) of the chains H and L.

In fact, an immunogen may contain several different epitopes capable of stimulating the synthesis of several antibodies of different specificity which forms a polyclonal antiserum.

1.2. Immunogen vs antigen

1.2.1. What subtlety does this definition hide? An immunogen is an antigen whereas the opposite is not necessarily true. Indeed, some molecules can react with an antibody but can not induce its synthesis. These molecules are *haptens*. To be an immunogen, the structure of the molecule must be complex: molecular weight, usually higher than 1kDa, and be genetically different from the recipient animal species, ie, which receives the immunogen ordinarily subcutaneously.

1.2.2. Let us illustrate this with an example: clenbuterol used as a doping agent but also to structure the muscle mass.

a. This clenbuterol (molecular weight of 277 Da) is not immunogenic, fortunately for athletes! It is a hapten that can become immunogenic by chemically coupling it to bovine serum albumin for example (BSA,

molecular weight: 60 kDa). The latter of high molecular weight and certain chemical complexity is immunogenic for different animal species other than bovines.

b. Let us consider the mouse as a recipient for the clenbuterol-BSA immunogen.

   The latter will synthesize IgM and then IgG during booster injections against different antigenic motifs, epitopes of BSA but also against clenbuterol that the immune system of the mouse has recognized as an epitope, part of the immunogen (BSA-clenbuterol). This hapten can therefore be an epitope that can react with a specific paratope.

2. Specificity and affinity: two essential properties of the antigen-antibody reaction.

2.1. The specificity

The specificity or, more precisely, the lack of specificity of an antibody is its ability to recognize a molecular structure similar to that against which it has been synthesized. This is called cross reaction. Take the example of anti-clenbuterol antibodies. They can also recognize other ß2 agonists such as salbutamol of close molecular structure.

2.2. Affinity

Affinity refers to the strength of the non-covalent binding that links the epitope of the Ag with the CDR (paratope) of the antibody. It is clear that the affinity depends in part on the specificity.

This reaction of the epitope with its corresponding paratope is a reversible reaction:

$Ag + Ab \leftrightarrow Ag\text{-}Ab$

This equation is governed by an equilibrium constant or affinity constant:

$Ka = (Ag\text{-}Ab) / (Ag) \times (Ab)$

This affinity constant is expressed in M^{-1}. For the high affinity Ab, the equilibrium is shifted to the right. This is the case for Ab whose Ka is equal to 10^8-10^{10} M^{-1}.

Another way of expressing the affinity of an Ag-Ab bond is to use the inverse of Ka, i.e. the dissociation constant (Kd): a high affinity antibody has a Kd = 10^{-8}-10^{-10}M. Then 1Kd = 10^{-9} M = 1nM.

Finally, the affinity characterizes the binding force of a single Fab fragment with its corresponding epitope. When we consider two Fab fragments of an

IgG, we speak instead of *avidity* which is greater than the sum of the affinities of the two Fab. It is the bonus effect, the rupture of the two bindings simultaneously is less likely than a single link.

Specificity and affinity constitute the main basis for the effectiveness of active and passive immunotherapy.

II. Active immunotherapy: vaccination

1. The origins: a brief reminder

The empirical practice of vaccination is ancient, much older than the objectification of its effectiveness by the scientific work of the past century. It exploits the two main characteristics of the reaction of an antigen with its corresponding antibody: specificity and affinity.

Curious about the origin of the term vaccination? It does not suggest an immune reaction, an antigen-antibody reaction. The word vaccine, and therefore vaccination, comes from the word vaccinia, the smallpox of cow, in Latin *vacca*. Smallpox (variole in French) itself originated from two Latin words: *varus* and *varius* meaning pustule and speckled, respectively. In fact, vaccinia in cows (cowpox) but also other animal species and smallpox in man are both characterized by speckled pustules.

In 1796, an English physician, Edward Jenner, objectified the phenomenon of variolation known since the Middle Ages, antiquity perhaps. He inoculated a young boy with the pus of a pustule taken from a dairymaid infected with cowpox, benign in man. He observed that this boy was later protected against human smallpox.

Vacca, varius, cowpox, smallpox, vaccine: vaccination was born.

Edward Jenner's experience, more than two centuries ago, is at the origin of the work of Louis Pasteur who in 1855 developed a vaccine against rabies. As for the discovery of Jenner, that of Louis Pasteur is based on the immunological cross reaction between two microbial strains: one virulent, the other not.

Thus, the work of these benefactors of humanity in the late eighteenth and the nineteenth centuries paved the way for prophylactic immunization against infectious diseases whose effectiveness led to the almost complete eradication in the twentieth century of many microbial, bacterial and viral diseases, once deadly.

2. Immune reaction and vaccination

2.1. The efficacy of a vaccine, by the administration of an immunogen, is based on a reaction at two levels, but interdependent as seen previously.

First, the triggering of the innate, so-called natural immune response. Then and mainly, the clonal proliferation and memory effect that characterize the cellular and plasma phase of the specific immune response, so-called acquired.

A first contact with the immunogen, whose description of the characteristics appears in the first part of the present work, leads to the synthesis of IgM (the receptor on the surface of B lymphocytes) followed by a discrete synthesis of IgG by plasma cells fellowed by clonal proliferation and memory T and B cells development.

2.2. In a booster with the same immunogen, the latency required for IgG synthesis is shorter (memory effect) while the IgM profile remains similar. Clonal proliferation of plasma cells is responsible for more sustained, higher IgG synthesis and improved affinity. These different characteristics contribute to optimal protection during repeated contacts with the pathogen in our environment or during the other injections during the vaccination process.

This synthesis of IgG does not depend only on the B lymphocytes but also on the T cells and APC that support the cellular immune reaction. The use of an adjuvant stimulates the innate and the cellular phase of the immune reaction.

3. Nature of Immunogens

3.1. Inactivated microorganisms

After culture on different types of milieus, the microorganisms, viruses or bacteria, are inactivated by a chemical (e.g. formaldehyde) or a physical (e.g. heat) agent. This inactivation process results in a loss of virulence but not immunogenicity that remains similar or identical to that of living pathogens.

For example, vaccines available include Salk anti-polio vaccine, anti-cholera vaccine and anti-rabies vaccine.

3.2. Live microorganisms attenuated

In this case, the vaccine is a living immunogenic microorganism similar or identical to the pathogenic strain but devoid of virulence.

Several attenuation methods exist for the preparation of such vaccines.

There are two examples available in Canada:

- The use of a heterogeneous virulent strain for another species but not for humans. This is the case in cowpox virus, which is effective in eradicating smallpox.
- Bacillus Calmette and Guérin (BCG), named after their discoverers, is a strain of Mycobacterium bovis made non-virulent by the growing conditions.

The live attenuated strain vaccine elicits a high cytotoxic T cell ($CD8^+$) response and sustains the antigenic stimulation.

The major but rare problem associated with an attenuated microbial strain is the minimal possibility that this strain regains its virulence by a retro-mutation, which was the case with the Sabin anti-polyomyelitis attenuated virus.

3.3. Toxoids

Toxoids are inactivated toxins. This is the case of the vaccine against tetanus or against diphtheria. The toxoids used are devoid of toxicity but have an immunogenicity identical to the native toxin.

3.4. Bacterial oligosaccharides

The polysaccharides of Hemophilus influenza and Streptococcus pneumoniae are used to vaccinate against these two microbial strains.

As mentioned previously, capsular polysaccharides are poorly antigenic.

In order to overcome this deficiency, these structures are thus coupled to a protein (tetanus toxoid, CRM 197 e.g.) which serves as a vector. This type of vaccine is however not recognized by the MHC class I-$CD8^+$ T lymphocyte complex.

3.5. Recombinant DNA

Vaccines synthesized by the recombinant DNA method are used to prevent hepatitis B and cancer associated with papilloma virus.

Hepatitis B vaccine is the recombinant surface antigen of the virus (HBs Ag) produced in yeast cells.

Vaccination against papilloma virus (HPV, human papilloma virus) developed to prevent cancer of the uterus also uses recombinant viral proteins representative of two, four or nine strains of HPV.

This latter approach opened a field of research aimed at developing vaccines using viruses or plasmids as vectors of the DNA encoding the antigen of interest.

3.6. Vaccines using mRNA

The injection of messenger RNA coding for a specific protein induces the synthesis of this protein immunogen in vivo.

This way of doing short circuits the nuclear DNA and takes advantage of the protein translation of the mRNA injected into the cytoplasm of the host APC.

Unlike the traditional vaccines described above, synthesis of a vaccine using messenger RNA is rapid. This explains the success of anti-SARS-CoV-2 vaccination campaigns in the fight against COVID 19.

However, the speed of producing such a vaccine is not the result of serendipity. On the contrary, it is based on extensive research over three decades devoted mainly to the following challenges. On the one hand, the synthesis and purification of a specific and functional mRNA sequence, resistant to in vivo metabolism. On the other hand, the vectorization of this mRNA molecule for its capture by the APC, most often by negatively charged nanoparticles, towards the APC, the an essential relay towards the specific immune reaction.

An excellent reference to learn more:

- Krammer F. SARS-CoV-2 vaccines in development. Nature. 2020 ;586:516-527. doi: 10.1038/s41586-020-2798-3.

III. Passive Immunotherapy

1. Maternal antibodies

During pregnancy, placental transport of maternal IgG via the *FcRn* receptor on the surface of placenta syncytiotrophoblasts confers passive fetal immunoprotection to the fetus. A similar phenomenon, mediated by the same receptor, continues after birth by intestinal absorption of maternal cholostrum IgG. On the other hand, maternal milk IgA present as dimer (SIgA) provide intestinal protection for the newborn.

2. Serotherapy

In 1888, Emile Roux and Alexandre Yersin isolate the diphtheria toxin they will use to develop an immune serum in horses.

In 1890, Emil von Behring and Danshaku Kitasato show that the protective power of antiserum developed in the horse against diphtheria toxin or tetanus is transferable thanks to the antibodies it contains.

These experiments open the door to passive immunotherapy. This consists in administering specific antibodies which confer to the patient a momentary and transient protection associated with the half-life of the IgG. These antibodies are provided by hyperimmune human or animal (horse) serum.

The administration of these immune sera is not deprived of side effects, mainly type I hypersensitivity reactions, also known as immediate or anaphylactic hypersensitivity, and type III hypersensitivity to immune complexes.

Currently, immune sera or IgG of human origin or humanized monoclonal antibodies are preferably used.

IV. Passive and specific immunotherapy: Monoclonal antibodies: Definition, production, nomenclature

1. Definition and production

1.1. Definition

A monoclonal antibody is an immunoglobulin, most commonly an IgG, whose paratope has a specificity and a well-defined affinity for a single epitope.

This monoclonal IgG is itself produced by a clone of all identical plasmocytes, derived from a single B lymphocyte.

As it is virtually impossible to isolate a monoclonal IgG from polyclonal immunoglobulins in an immune serum, it must be produced.

1.2. Production

Two main techniques are used in most monoclonal antibody producing laboratories: the hybridoma technique and the lymphoblastoid transformation technique by Epstein Barr virus (EBV). We describe the hybridoma method which is the most used.

This method was developed by George Köhler and César Milstein in 1975. This technique makes it possible to produce a monoclonal antibodys mAb) in vitro from a hybridoma resulting from the fusion between an immortal myeloma cell and a murine B plasma cell. This experimental approach involves the following steps:

1.2.1. The first step, in vivo, consists of immunization (vaccination) of a mouse using the immunogen against which it is desired to obtain one or more monoclonal antibodies depending on the number of epitopes. This immunization usually involves an adjuvant that potentiates the presentation of the immunogen and the proliferation of T cells.

1.2.2. The second step, after one or more boosts, consists of performing an in vitro fusion of B plasmocytes isolated from the spleen with immortal cells of a myelomatous line. Hybrid fused cells are called hybridomas. These are selected under specific culture conditions that eliminates both types of parental cells.

The third step is the selection of the hybridoma of interest, secreting the IgG of desired specificity and affinity.

This immortal hybridoma is amplified either by culturing it in vitro in a bioreactor system or by production of an ascitic fluid in the Balb/c mouse.

This immortal hybridoma can be frozen for future Mab production.

2. Use of monoclonal antibodies

Monoclonal antibodies were first produced for diagnostic purposes. They have proved to be a powerful analytical tool in vitro for the immunological assays of exogenous (e.g. drugs) or endogenous molecules (e.g. tumor markers). In vivo, they allowed targeting methods such as immunoscintigraphy.

The therapeutic use of these same monoclonal antibodies constitutes a field of application in full development.

First used to prevent rejection reactions after organ transplantation, they represent a major advance in the treatment of several diseases such as cancer or autoimmune diseases.

The side effects encountered in vivo, however, have limited the use for immunotherapy purposes.

In fact, a mouse monoclonal IgG injected into a patient is recognized as foreign, as non-self, by the patient's immune system and will be inactivted. In other words, this monoclonal antibody, in addition to its desired therapeutic effect, behaves as a antigen stimulating the synthesis of human IgM and then IgG anti-mouse IgG (HAMA: Human Anti Mouse Antibody) during injections in a chronic treatment, for example. These are present in some 80% of patients treated with mouse monoclonal antibodies. These immunization phenomena are accompanied by deposits of Ag-Ab complexes that underlie hypersensitivity reactions as horse serum used in serotherapy.

Technological advances in the field of molecular biology and bioinformatics have allowed rapid development of new types of monoclonal IgG closer to human antibodies and thus increased biocompatibility associated with a lower incidence of HAMA. Monoclonal antibodies of mice have been progressively modified into chimeric, humanized and finally human antibodies.

3. Monoclonal antibodies bio ingeniering.

3.1. Chimeric monoclonal antibodies

As the name suggests, a chimeric antibody is composed of two parts from two different animal species. The combination of murine variable domains and human constant domains is the most widespread but other combinations are also feasible.

Chimeric antibodies are obtained by grafting the constant parts H and L of human IgG on the variable parts H and L of a mouse monoclonal antibody. In this case, the variable part (30% of the Ig) which contains the Fab

fragment is of murine origin while the effector or constant part (Fc, 70% of the Ig) is human.

This monoclonal antibody thus has the specificity and affinity of the murine antibody obtained as described above.

Obtaining such antibodies makes use of molecular biology techniques: cloning and fusion of the DNA coding respectively for the V murine and C human parts before the expression of the chimeric monoclonal IgG in vitro.

3.2. Humanized monoclonal antibodies

The synthesis of humanized monoclonal antibodies is an advance in the search for biocompatibility. Indeed, not only the parts C of the H and L chains are human, but also the framework of the variable parts, i.e. the amino acid sequences surrounding the hypervariable parts which form the CDR. In this case more than 90% of the structure is of human origin.

In addition to molecular biology techniques, the synthesis of such humanized antibodies also requires computer tools to model the future humanized antibody in 3D structure to optimize the properties of the CDR while taking into account the nature of the adjacent amino acids (SDR: specificity determination residues) that contribute to the stability of the Fab fragments.

3.3. Human monoclonal antibodies

More recently, two new techniques have made it possible to completely get rid of the murine part of the monoclonal antibodies and to obtain completely human monoclonal antibodies.

The hybridoma technique being difficult to implement in the case of human cells, other techniques have been introduced: transgenic mice and "phage display".

The most frequently used method, that of "XenoMouse", is a transgenic mouse that express human IgG.

In a schematic manner, the first step is to inactivate genes coding for murine Ig in a mouse embryonic stem cell and to transfect this cell with DNA encoding human IgG.

The following steps are similar to those described above for obtaining the monoclonal antibodies: immortalization of the transfected cell by fusion with a myeloma cell, culturing, selection of the clone of interest, expansion for production of human monoclonal IgG and preservation by freezing.

The phage display technique consists in expressing (display) the antibody fragments on the surface of a filamentous phage in fusion with a protein of the capsid of the latter.

The starting point of the phage display technique is a classic in vivo immunization scheme. It leads to the production of plasma cells from which the RNA is extracted. It is used for the synthesis of cDNA.

These cDNAs are integrated into the DNA of a filamentous phage M13. The antibody fragments are coexpressed on the surface of the phage with the capsid protein pIII.

A strain of E Coli is then transfected with the bacteriophage bank which allows them to multiply.

The phages of interest i.e. expressing the antibody fragment of desired affinity are then detected and selected by an antibody-antigen reaction of the ELISA type in heterogeneous phase.

4. Nomenclature of monoclonal antibodies

The nomenclature of monoclonal antibodies has given rise to several revisions and was established by two WHO directives, one in 2014 (http://www.who.int/,medicines/Services/inn/BioRev2014. pdf), the other in 2017 (http://www.who.int/,medicines/Services/inn/meetings/eng/). Two more recent revisions respectively in 2021 and 2022 have made additional changes to this nomenclature.

For the sake of clarity, we will limit ourselves to the recommendations of the two directives of 2014 and 2017. Where appropriate and for specific cases, we will use the recommendations adopted in the 2021 and 2022 revisions.

This nomenclature of monoclonal antibodies is at first confusing, however, it is logical.

- The nomenclature of 2014 tells us about the nature of the monoclonal antibody, its origin and its therapeutic target.

In order, from right to left, we can distinguish:

- The suffix "mab" for monoclonal antibody. This suffix is the same for all monoclonal, chimeric, humanized or human antibodies.
- The first prefix, to the left of the suffix mab indicates the origin of this mab, the way it was synthesized.

Prefix	**For**
-o-	Mouse
-u-	Human
-xi-	Chimeric
-zu-	Humanised
-axo-	Hybrid rat/mouse

Examples

- xi-mab: chimeric monoclonal antibody (mab) (xi)
- zu-mab: humanized monoclonal antibody (mab) (zu)

- In anticipation of future developments, the international nomenclature has provided for other prefixes (!).

- The second prefix to the left of mab indicates the therapeutic target of this monoclonal antibody.

Prefix	**Target**
-tu-	Tumor
-li-	Immune system
-ci-	Cardiovascular system
-ki-	Interleukin
-vi-	Virus
-so-	Bone

- The third prefix to the left of mab is left to the discretion of the manufacturer.

- The revised nomenclature of 2017 modifies this one of 2014.

- he suffix mab is unchanged.

- The first prefix, the source of the antibody is discontinued for the new monoclonal antibodies not yet aproved.

- The nature of the second prefix is defined as felow:

Prefix	**Target**
-ami	Serum amyloid protein (SAP, amyloidose)
-ba-	Bacteria
-ci-	Cardiovascular
-fung-	Fungal
-gros-	Skeletal growth factors and their receptors
-ki-	Interleukin
-li-	Immunomodulating
-ne-	Neuronal
-os-	Bone
-toxa-	Toxin
-tu-	Tumor
-vet-	Veterinary
-vi-	Viral

Examples

- tuximab: chimeric (xi) monoclonal antibody (mab) targeting a tumor (tu).
- lizumab: humanized (zu) monoclonal antibody (mab) targeting the immune system (li).

- The third prefix on the left is said a fanciful prefix (sic!). It is left to the discretion of the manufacturer.

  Examples

  - Ce-tu-xi-mab: chimeric monoclonal antibody (mab) (xi) targeting a tumor (tu).
  - Toci-li-zu-mab: humanized monoclonal antibody (mab) (zu) targeting the immune system (li).

  This nomenclature, however, requires some remarks (As an example: Parrent P.W. et al.Mabs 2017, 9: 898-906.).

V. The monoclonal antibodies used for passive and specific immunotherapy: an inventory

It is in a didactic concern that this inventory has been drawn up.

To try to achieve this objective:

Based on the nomenclature defined above, we classified these antibodies according to their therapeutic target (idiotype). For each idiotype, we have defined the different isotypes approved by the FDA. The dissociation constant (Kd), inverse of the affinity constant, of the Ag-Ac complex formed by the monoclonal Ac and an epitope of its therapeutic target is of the nM range.

The light chain isotype does not appear to influence the effector properties of the antibody. In the majority of cases, it is of type κ.

The heavy chain isotype of the antibodies approved to date is of the γ type.

The isoform (IgG1-IgG4) of the monoclonal antibody is decisive for ensuring the effectiveness (effector properties) of the antibody linked to the Fc receptor.

The isoform and its possible modifications (mutations, deletions) are selected by the manufacturer according to the therapeutic effectiveness sought for each monoclonal antibody. They are mentioned in the text.

Finally, monoclonal antibodies as well as new targets having received the green light from the FDA in 2023 are indicated in green

1. Target: The cadiovascular domain (xxCIxxMAB)

Anti VEGF-A[1, 2, 3, 4]

Definition of the therapeutic target

Vascular endothelium growth factor A (VEGF-A) commonly referred to as VEGF, is one of the seven members of the VEGF family. VEGF is a growth factor produced by various cells, macrophages, platelets and tumor cells. It acts mainly on endothelil cells, stimulating the proliferation, migration and survival, thus leading to new blood vessel formation in the ambryo or in diseased tissues. VEGF also increase the permeability of blood vessels by uncoupling endothelial cell-cell junctions leading to tissue edema and extravasation of neoplastic cells for instance.

VEGF-A, of which there are different isoforms resulting from an alternative splicing, is a homodimer which exerts its vasodilating and angiogenic activities by stimulating mainly two types of endothelial receptors, VEGFR1 and VEGFR2 endowed with tyrosine kinase properties. It is mainly VEGFR2 which mediates the anti apoptotic properties of VEGF-A and its essential role in the mito-genesis, migration and hyper-permeability inside diseased or neoplastic tissues.

Thus, VEGF-A as well as its receptors play a central role in neovascularisation of ischemic retina. The new blood vessels may arise from the choroidal circulation in age-related macular degeneration or from the retinal circulation in proliferative diabetic retinopathy or ischemic retinal vein occlusion. These new blood vessels that develop as a healing response in non-neoplastic tissue result rather in damage of the ischemic retina by bleeding, leaking and leading to fibro-vascular scar formation. The hyper-permeability of the congenital retinal vessels may also cause macular edema which also leads to loss of central vision.

Anti VEGF-A strategies in cancer aim to inhibit angiogenic functions in tumor growth and metastases. They are indicated in advanced gastric and gastrointestinal adenocarcinomas after first line chemotherapy.

In age-related macular degeneration, diabetic retinopathy and retinal vein occlusions, the inhibition of VEGF-A aims to arrest ocular angiogenesis and reduce retinal edema, helping to preserve vision.

Monoclonal antibodies

- Ramucirumab (Cyramza, Eli Lilly), humanized IgG1. It antagonizes VEGF by binding to the extravascular domain of VEGFR2 with an affinity of the order of picomole.

- Bevacizumab (Avastin, Roche), humanized IgG1, the first VEGF-A inhibitor.
- Biosimilar: Bevacizumab (MVASI, Amgen), humanized IgG1.
- Ranibizumab (Lucentis, Genentech, Novartis), Fab fragment of bevacizumab.
- Brolucizumab (Beovu, Novartis), humanized ScFv.
- A *single chain variable fragment* (ScFv) is in fact a single-stranded protein resulting from the fusion of the VH and VL sequences of an Ig, connected by a peptide sequence of 25 aminoacids. It is therefore not an antibody fragment lacking the Fc fragment. Its molecular mass of only 26 kDa facilitates intra-tissue penetration.

A little history...

In an article entitled Therapeutic antibodies in ophthalmology old is new again (mabs 2010; 22: 176-180) Charlotte Magdelaine-Beuzelin reminds us that Henri Coppez, Belgian ophthalmologist, was the first in 1895 to use the anti-diphtheria antiserum developed by Roux in 1894 to treat diphtheria of the conjunctiva, systemically but also locally by conjunctival infusion.

The same Henri Coppez with his colleague Marcel Danis was also the first in 1923, a century ago, to describe age-related exudative macular degeneration. In 2006, the first anti VEGF-A monoclonal antibody was approved by the FDA.

Anti VEGF-A and anti ANGIOPOIETIN [5, 6]

Définition of the therapeutic target

Angiopoietin 2 (Ang-2) is one of the four angiopoietin family members. It is a growth factor playing an important role in angiogenesis. Isoforms 1 and 2, the most studied are respectively agonist and antagonist for an endothelial protein tyrosine kinase receptor (Tie-2)

Together with VEGF, Ang-2 contributes to the development, maturation and permeability of the vascular endothelium. They also play a key role in pathological angiogenesis and the alteration of vascular permeability characteristic of diabetic retinopathy and in age related macular degeneration (ARM).

Like VEGF, Ang-2 is expressed by smooth muscle cells and pericytes surrounding the vascular endothelium and regulates vasculogenesis. It acts in a paracrine way on the latter.

The bispecific monoclonal antibody is a heterodimer capable of simultaneously binding to Ang-2 and VEGF. It is used to prevent and suppress endothelial proliferation, neovascularization and vascular permeability associated with retinal thickening in ARM and DME (diabetic macular edema).

Monoclonal antibody

- Faricimab-svoa (Vabysmo, Genentech), Bispecific humanized IgG1 capable of simultaneously binding Ang-2 and VEGF-A.

Anti GPIIb / IIIa[7, 8]

Definition of the therapeutic target

Glycoprotein (GP) IIb / IIIa (CD41 / CD61) is an integrin expressed on the membrane of platelets.

It is responsible for platelet aggregation when subjected to the action of various activators (thrombin, collagen, ADP for example). This activation leads to the binding of coagulation factors including fibrinogen responsible for clot formation.

Anti GPIIb/IIIa antibody is used to prevent cardiac complications during percutaneous angioplasty. In unstable patients, it reduces rate of myocardial infarction and mortality.

The monoclonal antibody

- Abciximab (Reopro, Centocor Ortho Biotech), chimeric IgG1.

The Fab fragment of a mouse monoclonal antibody anti GPIIb / IIIa obtained by immunization against human platelets. This explains the lack of specificity of this antibody when compared to synthetic inhibitors (eg: tirofiban).

IgG1 and the FcRn receptor

As you will notice, several monoclonal antibodies belong to the IgG1 isoform.

How to explain this success?

First of all, by different characteristics specific to this isoform which facilitate its production and purification from culture media.

Then, by its biological effectiveness:

- Ability to activate the classical complement pathway and therefore CDC (complement dependent cytotoxicity via MAC) and ADCP (antibody dependent cell phagocytosis via C3b).

- Ability to activate ADCC (antibody dependent cell cytotoxicity via its Fc fragment).

Finally, by its long half-life:

Indeed, the IgG1 isotype has a half-life close to or equal to 21 days. The length of this contrasts with the short half-life of other Igs, such as IgG3: 7 days and IgE: a few hours.

How to explain this half-life equal to 3 weeks?

By the affinity of IgG1 for the FcRn receptor.

What does that mean?

The so-called neonatal FcRn receptor is different, both in structure and function, from FcγR receptors. It is not only responsible for passive immunoprotection of the fetus and newborn from maternal blood. It is also present for life in endothelial cells but also in epithelial cells in many tissues. FcRn is therefore a ubiquitous receptor responsible for the transcytosis of certain Ig isoforms, especially IgG1, IgG2 and IgG4 but also of albumin. The affinity of IgG1, IgG2 and IgG4 for this receptor is far superior to that of other isotypes and isoforms.

The FcRn receptor has a structure similar to that of MHC type I molecules, a heterodimer consisting of a ß2 microglobulin molecule associated with a 40kDa protein. However, it cannot present the antigen as an APC.

Once the plasma IgG1 molecule is absorbed by endothelial pinocytosis, it is taken up by the endosomal FcRn receptor. This binding via the interface between the CH2 and CH3 domains of the H chain of IgG1 is pH dependent. Indeed, the endosomal pH below 6.5 is essential for the protonation of 2 histidine residues (His 310 and His 435) responsible for the binding of IgG1 with the FcRn receptor. This connection has two consequences. On the one hand, it preserves IgG1 from lysosomal degradation; on the other hand, it recycles this same IgG1. Indeed, after transcytosis, the IgG1 molecule returns to the plasma compartment whose pH greater than 7 no longer allows this protonation of the 2 histidine residues of the IgG1 molecule.

This FcRn receptor finds all its interest in the development of monoclonal antibodies whose Fc fragment has an increased affinity for this receptor in order to increase its half-life and to space out its administration. (J. Immunol.2015; 194: 4595-4603)

On the other hand, it is currently the target for a monoclonal antibody used in the treatment of myastenia gravis. We'll talk about this again on page 81.

Anti PCSK9 [9, 10, 11, 12]

Definition of the therapeutic target

Proprotein convertase subtilisin / hexine type 9 (PCSK9) is a serine protease involved in the activation and inactivation of other enzymes and growth factors.

It is expressed mainly but not exclusively in the liver and secreted in the circulating blood.

PCSK9 plays an important role in the pathophysiology of familial hypercholesterolemia. Indeed, an autosomal dominant mutation associated with a gain in function is supposed to be responsible for the abnormality of its physiological role. PCSK9 binds to receptors for low density lipoproteins (LDL-R) causing lysosomal degradation of this complex (PCSK9-LDL-R) in the hepatocytes. This results in absence or reduced recycling of these receptors and therefore less clearance of cholesterol and hypercholesterolemia associated with circulating LDL.

The anti PCSK9 monoclonal antibody binds to this convertase in the plasma preventing the formation of the PCSK9-LDL-R complex in the liver. It is used for the treatment of familial hypercholesterolaemia, an autosomal dominant disease, associated with early cardiovascular morbidity and mortality.

Monoclonal antibodies

- Evolocumab (Repatha, Amgen), human IgG2 λ.
- Alirocumab (Praluent, Sanofi-Aventis), human IgG1.

Anti Angioprotéine –like 3 [13, 14]

Definition of the therapeutic target

Angiopoietin-like 3 (ANGTL3) is a member of the family of the same name which currently has 8 ANGTL: ANGPL1 to ANGPTL8 similar in structure to the angiopoietins previously described.

ANGPTL3 is a protein synthesized in the liver. It consists of 3 distinct domains.

The pathophysiological role of each of these ANGPTL in humans has not been fully elucidated. Studies in laboratory animals have shown that

ANGPTL and in particular ANGPTL3 are involved in plasma lipid metabolism.

ANGPTL3 increases plasma triglycerides by inhibiting lipoprotein lipase and endothelial lipase and stimulating adipose tissue lipolysis. A deficiency of ANGPTL is accompanied by hypo triglyceridemia and a significant decrease of total cholesterol and plasma LDL levels accompanied by a lower risk of cardiovascular disease with no apparent effect on CHO-HDL.

The formation of an immune complex between the monoclonal antibody and ANGPL3 leads to an increased metabolism of LDL.

Monoclonal antibody

- Evinakumab (Evkeeza, Regeneron pharmaceutica), human IgG4.

Prophylactic treatment of hemophilia A [15, 16, 17]

Definition of the therapeutic target

Hemophilia A, an inherited disease transmitted by the X chromosome, is caused by a congenital factor VIII deficiency responsible for an abnormality in primary hemostasis responsible for life-threatening hemorrhages.

Coagulation factor VIII is a plasma glycoprotein synthesized primarily by the liver. During coagulation, it is activated by thrombin in factor VIIIa, a cofactor of factor IXa for the activation of factor X in the presence of phospholipids. Factor Xa in turn transforms prothrombin into thrombin which itself generates fibrin from fibrinogen.

The role of the monoclonal antibody, substitute of factor VIII, is to bring factor IXa (enzyme) into contact with factor X (substrate) and thus correct factor VIII deficiency and prevent bleeding.

Monoclonal antibody

- Emicizumab (Hemlibra, Roche), humanized IgG4 bispecific : the anti factor IXa Fab fragment is rat, the other, anti factor X, is mouse.

Anti Dabigratan [18, 19]

Definition of the therapeutic target

Dabigratan is an anticoagulant drug that inhibits thrombin directly.

The Fab fragment of a monoclonal antibody binds to dabigratan with a higher affinity than that for thrombin, thereby inhibiting its anticoagulant power and preventing hemorrhages, in the case of an overdose, for example.

Monoclonal antibody

- Idarucizumab (Praxbind, Boeringher Ingelheim), humanized IgG1 Fab fragment.

By its molecular mass (471 Da), dabigratan is a hapten, devoid of immunogenic power, the obtaining of a specific monoclonal antibody in mouse therefore required its coupling with an immunogenic vector protein, bovine serum albumin for example.

Anti von Willebrand factor [20, 21, 22]

Definition of the therapeutic target

Von Willebrand factor (vWF) is a multimeric glycoprotein synthesized by endothelial cells and megakariocytes. Each monomer, of variable molecular weight, contains several domains (A-E)

Through its domain A, vWF interacts with the GPIb receptor on the surface of platelets. It is therefore essential for their adhesion and aggregation in contact with the injured blood vessels.

The monoclonal antibody, a *nanobody,* is used for the treatment of thrombotic thrombocytopenic purpura caused by a lack of activity of ADAM ST 13, the metalloprotease responsible for the proteolysis and activation of multimeric vWF. This autoimmune enzyme deficit results in the formation of platelet microthrombi in the arterioles and capillaries in the absence of vascular damage. The monoclonal antibody against the A domain of multimeric vWF inhibits its interaction with platelets.

Monoclonal antibody

- Caplacizumab (Cablivi, Ablynx Sanofi), humanized nanobody.

Do more with less...

A nanobody (e.g. caplacizumab) has a lower molecular weight (12-15 kDa) than the Fab fragments (50kDa) and scFv (27kDa). It is the smallest therapeutic monoclonal antibody fragment currently approved by the FDA.

These different fragments obtained by bioengineering have been developed to increase their tissue penetration but also the reaction of their paratope with a cryptic epitope, difficult to access for a monoclonal IgG (150kDa). Deprived of the Fc fragment, they cannot however benefit from ADCC (antibody dependent cell mediated cytotoxicity) for their therapeutic efficacy. In addition, their low molecular mass, in any case for scFv and nanobodies, allows their glomerular filtration and their renal excretion responsible for a lower blood and tissue concentration and therefore for closer administration.

A little word now about nanobodies

These nanobodies are the result of serendipity much like the discovery of penicillin.

Indeed, almost 30 years ago, Dr C. Hamers-Casterman and his collaborators at the Vrije Universiteit te Brussels in Belgium (Nature 1993; 363: 446-448) show that the plasma of Camelids (camel, dromedary, lama ...) contains, of course, the classic IgG of tetrameric nature but also Ig of lower molecular mass, deprived of light chains and composed only of two heavy chains. These heavy chains are themselves composed of two constant domains (CH2 and CH3), a hinge region and a variable part called VHH. The latter includes 3 CDR capable of binding to a specific epitope.

Humanized nanobodies, including caplacizumab, are made up of these 3 humanized lama CDR. Obtaining them is an application of the phage display.

2. Target: The interleukins (xxKIxxMAB)

Anti IL-1beta [23, 24, 25]

Definition of the therapeutic target

Together with interleukin Iα (IL-1α), IL-1ß is the most studied and best known among the 11 members of the IL-1 family. IL-1α and IL-1ß show a high degree of homology as regards their structure and their activity; however, the most important from a pathophysiological point of view is IL-1 ß.

It is released from a membrane precursor (33 kDa), proIL-1ß, by caspase 1 of monocytes, macrophages, polynuclear neutrophil and dendritic cells in response to stimulation by a DAMP or a PAMP. TNFα can also stimulate the formation and secretion of IL-1ß.

IL-1ß (17 kDa), also known as endogenous pyrogen, is an important mediator of the inflammatory reaction (non-specific immune response) both locally and systemically. Its activity profile is similar to that of TNFα.

IL-1ß is an essential mediator of the natural immune response both by its pro-inflammatory properties similar to those of TNFα and by its receptor analog of the Toll receptors expressed on the surface of different cells, endothelial cells or leukocytes, for example. Like IL-6 and TNFα, it increases the expression of E selectin on the surface of post capillary endothelial cells, integrins and leukocyte chemokines thus promoting adhesion, chemotaxis and transmigration of lood polynuclear cells and monocytes to the inflammatory site.

IL-1ß plays a central role in autoinflammatory diseases grouped under the term hereditary cryopyrinopathies or hereditary cryopyrin-associated periodic syndrome (CAPS), autoinflammatory diseases without infection or autoimmunity.

Anti IL-1ß monoclonal antibody is used in the treatment of autoinflammatory diseases as idiopathic juvenile arthritis. Its effectiveness is objectified by the normalization of blood parameters including CRP and the decrease in circulating IL-6 levels.

Monoclonal antibody

- Canakinumab (Ilaris, Novartis), humanized IgG1.

Anti IL-12 and anti IL-23 [26, 27, 28]

Definition of the therapeutic target

Interleukins 12 (IL-12) and 23 (IL-23) are two members of the IL-12 family.

IL-12 is a heterodimer cytokine composed of 2 protein subunits, p35 and p40. It shares the latter subunit with IL-23, also heterodimer, with the p19 subunit. They are synthesized and secreted mainly by dentritic cells and macrophages stimulated by a DAMP or a PAMP.

By their common subunit, p40, IL-12 and -23 activate a common heterodimeric receptor IL-12Rß1 and ß2. By its p19 subunit, IL-23 also activates a receptor which its own: IL-23Rα. The presence of these receptors on the surface of NK cells and T lymphocytes thus triggers cytotoxicity and the differentiation of CD4+ and CD8 +T lymphocytes. For example, IL-12 stimulates the production of INFγ and TNFα by CD4+Th1 lymphocytes while IL-23 stimulates the production of IL-17 by CD4+ Th17 lymphocytes.

IL-12 and IL-23 therefore contribute directly or indirectly to the recruitment and activity of different immune reaction cells (CD4+, CD8+ lymphocytes, NK cells) whose dysfunction characterizes autoimmune diseases. In these conditions, the concentration of the plasma p40 subunit common to IL-12 and IL-23 correlates with the severity of the disease.

The anti IL-12 monoclonal antibody binds to its p40 subunit, common with IL-23. Inhibition of these two cytokines blocks the formation of Th1 (IL-12) and Th17 (IL-23) lymphocytes.

The anti IL-23 antibody binds to its specific p19 subunit.

These antibodies are used to treat Crohn's disease, psoriasis and psoriatic arthritis.

Monoclonal antibodies

- Anti IL-12 and IL-23: Ustekinumab (Stelara, Centrocorps Ortho), humanized IgG1.
- Anti IL 23:
    - Guselkumab (Tremfya, Janssen), human IgG1λ.
    - Rizankizumab (Skyrizi, AbbVie), humanized IgG1.
    - Tildrakizumab (Ilumya, Merck), Humanized IgG1.
    - Mirikizumab (Omvoh, Eli Lilly) humanized IgG4. The hinge region was stabilized by S228P mutation. The effector functions of the Fc fragment were removed by mutation and deletion.

About IgG4…

Plasma IgG4 represents the least abundant isoform of the 4 isoforms of the IgG isotype, however it has a t1/2 similar to that of IgG1 and IgG2, the most abundant.

The nature of its Fc fragment explains the potential choice of a monoclonal IgG4 in the construction of different monoclonal antibodies when the effector functions of the Fc fragment are not required for therapeutic effectiveness. Indeed, the Fc fragment of IgG4 has a low affinity for thedifferent cellularFcγR receptors or for the complement factor C1q. The IgG4 subclass is therefore used when ADCC, ADCP or complement activation with MAC formation is not desired or required for therapeutic effectiveness.

However, the IgG4 subtype has another particularity: the ability to exchange half of its structure (a light chain and a heavy chain, or FAb arm exchange).

Thus, in solution in plasma, a monoclonal antibody molecule, for example mirikizumab which we have just spoken about, has the capacity to exchange one of its arms formed of Fc and Fab fragments with another circulating IgG4 molecule, present in the patient's blood, for example. This exchange has two consequences. The first consists of transforming the divalent mirikizumab molecule (two Fab fragments with the same affinity and specificity) into a monovalent hybrid IgG4 molecule.

The second and most important consequence for the effectiveness of immunotherapy: loss of avidity. As we have seen, avidity is due to the two paratopes of the mirikizumab molecule and is greater than the sum of the affinities of each of both paratopes (the bonus effect).

This exchange phenomenon is due to a rupture of the interchain disulfide bridges between the two H chains in the hinge region. To overcome this drawback, therapeutic monoclonal IgG4 is equipped, among other mutations (we will come back to this), with an S228P mutation.

Useful reference: Rispens T. and Huijbers M.G. Nat Rev Immunol. 2023;23:763-778. doi: 10.1038/s41577-023-00871-z.

Anti IL-17A and anti IL-17 Receptor (IL-17R) [29, 30, 31, 32]

Definition of the therapeutic target

Interleukin 17 (IL-17 or IL-17A) is a pro-inflammatory cytokine member of the IL-17 family of six (A to F). These cytokines have a certain degree of homology, higher for IL-17A and F. The circulating forms of IL-17 are either homodimers consisting of two chains IL-17A or IL-17F or a heterodimer composed of a chain IL-17A and one chain IL-17F.

Their synthesis mainly by $CD4^+$ Th17 lymphocytes is stimulated by IL-23.

IL-17 stimulates the recruitment and activation of neutrophils and macrophages in the inflammatory site. It works by stimulating its dimeric transmembrane receptor expressed on the surface of many cells, thus triggering the secretion of IL-8 involved in the recruitment of neutrophils. The concentration of IL-17 is high in the blood of patients with psoriasis in which it contributes to inflammatory symptoms.

Monoclonal antibodies inhibiting IL-17A or antagonizing (IL-17RA) its activity at its receptor level. It inhibits the recruitment of leukocytes at the level of the inflammatory site. They are used for the treatment of diseases which also serve as a target for anti IL-23 and anti IL-12 antibodies.

Monoclonal antibodies

- IL-17 antagonist:
    - Brodalumab (Siliq, Valeant), human IgG2.
- IL-17 inhibitors:
    - Ixekizumab (Talz, Lilly), humanized IgG4.
    - Secukinumab (Cosentix, Novartis), human IgG1.
    - Bimekinumab (Bimzelx, UCB pharma), humanized IgG1.

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Anti TSLP [33, 34, 35, 36]

Definition of the therapeutic target

TSLP (Thymic stromal lymphoprotein) is a proinflammatory 157 AA pleiotropic cytokine paralog of IL-7 implicated in the pathophysiology of atopic conditions such as dermatitis, asthma and food allergies.

It acts via a heterodimeric receptor formed of an IL-7R-α chain (CD 127) and a TSLPRγ chain expressed in different cell types. These include: dendritic cells, mast cells, macrophages, eosinophils and basophils, T and B lymphocytes, smooth muscle cells and cancer and NKT cells.

Epithelial cells and stromal cells are the main source of TSLP at the pulmonary, gastrointestinal and cutaneous level when subjected to the action of pro-inflammatory agents of a chemical, microbiological or viral nature. In the latter case the syncitial virus for example. Other cells can produce TSLP, including fibroblasts, basophils, and stimulated mast cells. Its synthesis is itself regulated by IL-4, IL-13, TNFα, IL-1ß and IgE, via the NFκB pathway.

TSLP is an alarmin, it promotes and amplifies immunity mediated by TH2 lymphocytes. It stimulates MHC-Class II dendritic cells and induces the synthesis of CD80, CD86 and the ligand OX40 which in turn induce the proliferation of naive CD4+ T lymphocytes and their differentiation into TH2 lymphocytes producing allergenic cytokines IL-4 and IL-5, IL-13 and TNFα responsible for IgE synthesis. IL-10 like corticosteroids inhibits its synthesis and its release.

TSLP is highly expressed in atopic dermatitis lesions. Patients with atopic asthma have elevated THSLP and cytokine levels in the airways correlating with severity.

The monoclonal antibody blocks the binding of TSLP to its receptor, reducing the inflammation associated with eosinophils, thus reducing the exacerbation of the disease in asthmatic patients and controlling asthma attacks.

Monoclonal antibody

- Tezepelumab (Tespire, Astra Zeneca), human IgG2λ.

3. Target: Immune reaction (xxLIxxMAB)

Anti TNF alpha [37, 38. 39, 40]

Definition and therapeutic target

The tumor necrosis factor alpha (TNFα) is a powerful proinflammatory and pluripotent cytokine member of the TNF superfamily. It is synthesized in a transmembrane form (26 kDa) by very different cells: monocytes, macrophages, polynuclear neutrophils, activated NK cells, certain T lymphocytes, keratinocytes, microglial cells of the central nervous system. This form of TNFα is released from its membrane support by various enzymes including TACE (metalloproteinase TNFα converting enzyme).

Both transmembrane and circulating forms are biologically active.

They exercise their activities by stimulating two types of receptors: CD120a (TNFR1) and CD120b (TNFR2). They have a certain structural analogy and belong to the TNFR superfamily which has 40. TNFR1 is constitutive and ubiquitous whereas TNFR2 has a more restricted localization at the endothelial, neuronal and immune level. TNFR1 is activated by one and the other form of TNFα whereas TNFR2 whose role remains little known is activated by the transmembrane form.

The binding of TNFα to its receptor leads to the recruitment of TRAF (TNF receptor associated factor) which stimulates the activation of factors NF-κB, the caspase pathway, which leads to apoptosis.

TNFα has a dual action. At low concentration, alone or in collaboration with other cytokines and chemokines, it plays a regulatory role at the level of the two immune reactions and their interrelations but also in tissue repair. At high concentration, synthesized by monocytes and macrophages, locally and systematically, it has a deleterious action.

Prolonged and excessive activation of immune cells including B and T lymphocytes as well as increased and continuous synthesis of TNFα and other cytokines including IL-1 and -6 play a central role in the pathogenesis of the autoimmune diseases. Through its proinflammatory properties, TNFα therefore plays an important role in the progression of rheumatoid arthritis (e.g. secretion of metallopeptidases by macrophages, fibroblasts, osteoblasts and chondrocytes) and Crohn's disease. High concentrations of TNFα have been measured in the feces of Crohn's disease patients and in the joints and synovial fluid during rheumatoid arthritis.

At the systemic level, it acts on the hypothalamus to induce fever and at the liver level where it increases the synthesis of APP including CRP.

TNFα inhibitors are prescribed for the treatment of various rheumatic conditions (rheumatoid arthritis, juvenile arthritis, psoriatic arthritis, juvenile idiopathic arthritis, ankylosing spondylitis), Crohn's disease and ulcerative colitis. Their effectiveness is evaluated by clinical signs and is objectified by laboratory tests.

The rare side effects (infections, lymphomas, neurological complications) of anti TNFα monoclonal antibodies are explained by the role of this cytokine in the immune reaction.

Monoclonal antibodies

Currently, several monoclonal anti TNFα antibodies have been approved by the FDA. Their affinity has been defined. The crystallography made it possible to identify the epitope recognized by these various antibodies. Their effectiveness is similar in the treatment of rheumatic conditions but not in the treatment of Crohn's disease. They also have differences in their administration. The antibodies are:

- Adalimumab (Humira, Abott), humanized IgG1. This antibody binds to circulating and transmembrane TNFα. It inhibits the interaction of TNFα with its receptors, thereby blocking the synthesis of NF-κB-mediated cytokines.

  Its half-life is like that of natural plasma IgG1. Its affinity is subnanomolar. From the specific point of view, it does not recognize TNFß.

  Biosimilars: Amjevita (Amgen), human IgG1; Hadima (Samsung Bioepis), human IgG1.

- Infliximab (Inflacra, Hospitera), (Remicade, Centrocor Ortho Biotech, Johnson and Johnson), Chimeric IgG1.

  The indications are similar to those of adalimumab. Three biosimilars have been developed: Remsima (Biogaran, Servier), Inflectia (Hospitera Pfizer) and Renflexis (Merck).

- Golimumab (Simponi, Centrocor Ortho Biotech, Johnson and Johnson), human IgG1.

  Its affinity is higher than that of infliximab and adalimumab. Also, its affinity for soluble TNFα is greater than that for membrane TNFα.

- Certolizimab (Cimzia, UCB), Fab fragment of a humanized antibody devoid of its Fc fragment.

  The Fab fragment by its hinge region is pegilized i.e. connected to 2 chains of polyethylene glycol (PEG) of 20kDa to increase the half-life.

It activates neither complement nor ADCC, for lack of Fc fragment and this contrary to the complete monoclonal antibodies which activate the complement and therefore can lead to the opsonization of cells which express TNFα.

Note: Etanercept is not a monoclonal antibody but an immunoadhesin-like genetic fusion molecule. Unlike monoclonal antibodies, etanercept also recognizes lymphotoxin TNFß and forms an unstable complex with TNFα.

Anti IL-2R [41]

Definition of the therapeutic target

Interleukin 2 (IL-2) is an auto and paracrine cytokine secreted mainly by $CD4^+$ T lymphocytes but also to a lesser extent by $CD8^+$T lymphocytes, dendritic cells, NK and NKT. IL-2 is a growth factor for lymphocytes.

IL-2 exercises its physiological activities by stimulating its IL-2R receptor present on the surface of activated T lymphocytes and in particular T reg. IL-2R is composed of 3 subunits: α (CD25), ß (CD122) and γc (CD132). The binding of IL-2 to CD25, specific for IL-2 leads to the recruitment of the ß and γ chains, and to the formation of a trimeric receptor of high affinity, capable of intracellular signaling.

The antagonism of the α-chain (CD25) of the T-cell IL-2R receptor by a monoclonal antibody leads to a suppression of the immune reaction responsible for autoimmune phenomena and transplant rejection.

Monoclonal antibody

- Basilixumab (Simulect, Novartis), chimeric IgG1.

Anti IL-4R [42, 43, 44, 45, 46, 47]

Definition of the therapeutic target

Interleukin 4 (IL-4) is a pleiotropic cytokine involved in the regulation of the specific immune response.

It is secreted mainly by $CD4^{+}$Th2 lymphocytes, but also by mast cells, eosinophils and basophils.

Similar to IL-13, IL-4 participates with other cytokines, in the transformation of $CD4^{+}$T lymphocytes into Th2 in response to an allergen or a parasite, These activated lymphocytes proliferate and in turn produce IL-4, IL-5 and IL-13 responsible for eosinophilia and the differentiation of activated B cells into plasma cells producing IgE and IgG4.

Under the effect of IL-4, the α chain of its receptor (IL-4Rα) dimerizes with with a γ chain forming a type I signaling complex ((IL-4Rα / γ). This same α chain can also dimerize with an α chain of the IL-13 receptor to produce a type II complex (IL-4Rα / IL-13Rα) also expressed on non-hematopoietic cells, and responsible for the non-immune response to IL-4 and IL-13 such as bronchial hyperactivity and mucus production, for example.

Antagonizing the binding of IL-4 via the type I receptor but also IL-4 and IL-13 via the type II receptor is used in the treatment of allergies, eczema and atopic dermatitis.

Monoclonal antibody

- Dupilumab (Dupixent, Regeneron Pharm.), human IgG4.

Anti IL-5 and anti IL-5R [48, 49, 50]

Definition of the therapeutic target

Interleukin 5 (IL-5), a 134-amino acid homodimer, is produced primarily by $CD4^+$Th2 lymphocytes, but also by eosinophilic polynuclear cells and mast cells.

IL-5 activates T and B cells and in particular stimulates IgE production. IL5 is the main cytokine involved in the activation of eosinophilic polynuclear cells, playing a role in the pathogenesis of allergic diseases.

The membrane receptor of IL-5 (IL-5R) is expressed on the surface of eosinophilic and basophilic polynuclear cells. Heterodimer, it is composed of two subunits, α specific for IL-5 and β common with the IL-3 and granulocyte-macrophage colony stimulating factor (GM-CSF). The binding of IL-5 leads to dimerization of the receptor, intracellular signal transduction, inhibition of apoptosis and therefore survival and activation of eosinophilic polynuclear cells.

The monoclonal antibody inhibits or antagonizes the growth factor power of IL-5 on lymphocytes, eosinophilic and basophilic polynuclear cells and prevents eosinophilic asthma and atopic dermatitis.

Monoclonal antibodies

- IL-5 inhibitors:
  - Reslizumab (Cinqair, Teva), humanized IgG4.
  - Mepolizumab (Nucala, GlaxoSmithKline), humanized IgG1.
- IL-5 antagonist:
  - Benralizumab (Fasenra, AstraZeneca), human IgG1.

Anti IL-6R [51, 52, 53]

Definition of the therapeutic target

Interleukin 6 (IL-6) is a pleiotropic, proinflammatory cytokine.

Homodimer, it is synthesized by different cells in response to PAMP, IL-1 and TNFα: macrophages, dendritic cells, endothelial cells. It performs proinflammatory activities by stimulating a receptor (IL-6R, CD126) and a transmembrane protein gp 130.

IL-6 is endowed with local and systemic effects. It is involved in the differentiation of Th lymphocytes into Th17 and Threg, it stimulates the secretion of cytokines by endothelial cells and activates osteoblasts and RANKL expression.

The mab was first used for the treatment of Castelmann's disease. It is also uded in the treatment of rheumatoid arthritis.

More recently a monoclonal antibody has been developed against the IL-6 receptor. It is used for the treatment of neuromyelitis optica disorder (NMOSD) an autoimmune disorder of the CNS affecting the optic nerve associated with the presence of anti-aquaporin-4 autoantibodies.

Monoclonal antibodies

- Tocilizumab (Actemra, Roche), humanized IgG1.
- Sarilumab (Kevzara, Regeneron Sanofi), humanized IgG1.
- Siltuximab (Sylvant, Janssen), chimeric IgG1.
- Satralizumab (Enspryng, Roche), humanized IgG2

Anti IL-13 [54, 55]

Definition of the therapeutic target

IL-13 is a cytokine synthesized and secreted mainly by Th2 lymphocytes but also by other cells such as basophils, eosinophils and NK cells.

Its secondary but not primary structure has an analogy with that of IL-4.

IL-13 binds to the IL-13Rα1 subunit of its receptors on the surface of B lymphocytes and monocytes. This binding is followed by the recruitment of the IL-4Rα chain, followed by a dimerization responsible for the transduction of an intracellular signal leading to the differentiation of Th2 lymphocytes and a change of isotype (IgE) produced by B lymphocytes.

A circulating variant of IL-13Rα1 is IL-13Rα2 which is a decoy receptor.

The overproduction of IL-13 plays a central role similar to that of IL-4 in the initiation and maintenance of the inflammatory reaction associated with TH2 lymphocytes and the dysfunction of the epidermis characteristic of atopic dermatitis. Elevated concentrations of IL-13 have been measured in the characteristic lesions of this dermatitis. They are responsible for remodeling the epidermis and increase susceptibility to skin infections.

The anti IL-13 monoclonal antibody inhibits the binding of IL-13 to its receptor. It is used for the treatment of atopic dermatitis.

Monoclonal antibody

- Adtralza (Adbri, Medimmune and Léo Pharma), human IgG4.

Anti IL-36 [56]

Definition of the therapeutic target

IL-36 is an inflammatory cytokine member of the IL-1 family, IL 36 has 4 isoforms: 3 agonists α, ß and γ respectively and an antagonist IL-36-Ra

IL 36 is synthesized and expressed mainly by T lymphocytes but also other cells, including keratinocytes, in an inactive form that must be activated by leukocyte proteases. IL-36 induces the synthesis of proinflammatory cytokines and chemokines (IL-17C, G-CSF, IL-8 and TNFα) by activating an IL-36R but also IL-1RAcP.

IL-36R is a dimeric receptor triggering an inflammatory reaction via the activation of the MAPK and NFκB pathway of dendritic cells and keratinocytes.

In synergy with other cytokines, IL-36 is involved in the pathophysiology of systemic lupus erythematosus (SLE) and psoriasis, its serum concentration correlates with the level of activity of the disease.

The monoclonal antibody is used for the treatment of the generalized pustular psoriasis and particularly for the palmoplantar pustulosis.

Monoclonal antibody

- Spezolimab (Spevigo, Boehringer Ingelheim), humanized IgG1.

Anti BLYS [57, 58]

Definition of the therapeutic target

The B lymphocyte stimulator (BlyS) is a cytokine, a member of the TNF ligand family, also known by the acronym BAFF (B-lymphocyte activating factor).

It is released during the activation of different cells involved in immune reactions: monocytes, but also T cells and dendritic cells.

Only the soluble form of BLyS is active. It binds to 3 types of membrane receptors leading to the activation of NF-κB and to the expression of genes involved in B cell survival.

BLyS is a stimulator of B lymphocytes. It plays an important role in the development and differentiation of B lymphocytes into plasma cells and thus in the production of antibodies.

High levels of BLyS are found in SLE where it is involved in B cell activation and autoantibody synthesis.

Inhibition of BLyS prevents its binding to its receptor and limits the life of B lymphocytes, their differentiation into plasma cells and the production of autoantibodies. Used as an immunomodulator in the treatment of SLE, the level of anti-circulating DNA antibodies reflects its therapeutic efficacy.

Monoclonal antibody

- Belimumab (Benlysta, GlaxoSmithKline), human IgG1.

Anti CD 20 [59, 60]

Definition of the therapeutic target

CD20 is a phosphoprotein marker of differentiation of B lymphocytes.

Transmembrane calcium channel, CD20 is a homotetramer present on the surface of pre-B lymphocytes, but not their precursors, mature B cells or memory B lymphocytes but not on plasma cells. It is involved in the differentiation, activation and proliferation of normal and malignant B cells. It is not known to have an endogenous ligand, at least at present.

Anti CD20 antibody is used to deplete B lymphocytes. It finds its application in the treatment of autoimmune diseases characterized by the presence of dependent T monoclonal auto antibodies such as SLE and multiple sclerosis. In this case, the monoclonal IgG bands are detected in cerebrospinal fluid.

Monoclonal antibody

- Ocrelizumab (Ocrevus, Genentech), humanized IgG1.

Anti check points: CTLA4, PD1 and PDL1[61, 62, 63, 64, 65]

Definitions of the therapeutic targets

Two types of T lymphocyte coreceptors activation control points, among the many others described, are currently of therapeutic interest: CTLA-4 and PD-1. We have already described them, page 19.

CTLA-4 and PD-1 are two targets of interest for supressing the tolerance of tumor cells to T cell-dependent immune response by either blocking CTLA-4 and PD-1 receptors or inhibiting PD-L1. They are at the origin of the check point therapy.

The injection of monoclonal antibodies anti CTLA-4, anti PD-1, anti PD-L1 causes a depletion of Treg cells thus promoting the action of cytotoxic T lymphocytes ($CD8^+$) on the neoantigens of the tumor.

The simultaneous blocking of two targets (CTLA-4 and PD-1) are complementary and effective in the treatment of melanoma, lung carcinoma and Hodgkin's lymphoma. However, some patients do not respond to such PD-1 or / and CTLA-4.

Autoimmune side effects are predictable because of the role of PD-1 and CTLA-4 in the autotolerance and regulation of T-cell response.

Monoclonal antibodies

- Anti CTLA-4
  - Ipilimumab (Yervoy, Bristol Mayers Squibb), human IgG1.
  - Tremelimumab (Imjudo, Pfizer), human IgG2, alone or in association with durvalumab.
- Anti PD-1
  - Pembrolizumab (Keytruda, Merck), humanized IgG4.alone or with enfartumab vedotin (Padcev, Astellas pharma), anti nectin 4 human IgG1, an ADC.
  - Nivolumab (Opdivo, Bristol Myers Squibb), human IgG4.
  - Cemiplimab (Libtayo, Sanofi), human IgG4.
  - Dostarlimab (Jemperli, GSK), humanized IgG4.
  - Retifanlimab dlwz (Zynyz Incyte corp, Macrogenics, Zailab) ,humanized IgG4.
  - Toriplomab tpzi (Loqtorzi, Shanghai Junshi Inc et Coherus Biosciences), humanized IgG4.

- Anti PD-L1
    - Avelumab (Bavencio, Merck, Pfizer, Eli Lilly), human IgG1.
    - Durvalumab (Infinzi, Astrazeneca), human IgG1.
    - Atezolizumab (Tecentriq, Genentech Roche), humanized IgG1 with a N297A mutation responsible for the absence of Fc fragment glycosylation.

Anti LAG-3 [66, 67, 68]

Definition of the therapeutic target

LAG-3 is a checkpoint inhibitor that is part of the Ig superfamily with some homology to CD4.

LAG-3 is expressed on T lymphocytes and NK cells but also on B lymphocytes, dendritic cells and Treg lymphocytes. When bound to its ligand fibrinogen-like protein 1 (FGL1) on the surface of MHC class II DC, LAG-3 negatively regulates T cells and NK cells allowing tumor cells to escape to the immune response in combination with other checkpoints like PD1.

LAG-3 signaling impairs T cell proliferation, cytokine production and cytolytic function.

LAG-3 negatively correlates with proliferation of cancer cells: follicular lymphoma, acute myeloid leukemia, diffuse large B cell lymphoma or chronic lymphoid leukemia

The monoclonal antibody has be proposed for the treatment of chronic lymphocytic leukemia and melanoma.

Monoclonal antibody

- Relatimab (Opdualg ,BMS) , human IgG4.

Anti CD11a [69, 70, 71]

Definition of the therapeutic target

CD11a is the α chain of integrin αLß 2 also known as leucocyte function associated antigen 1 (LFA-1). Present on the surface of leukocytes, this integrin interacts with the intercellular adhesion molecule-1 (ICAM-1) present on the surface of endothelial cells and APC. It is essential for lymphopoiesis and in particular for the differentiation of $CD4^+$ and $CD8^+$ T lymphocytes.

The monoclonal antibody prevents the interaction of CD11a with ICAM-1. It thus blocks several steps of the immunological process involved in the pathogenesis of psoriasis. However, its efficacy is less than that of anti TNFα monoclonal antibodies.

Monoclonal antibody

- Efalizumab (Raptiva, Genentech), humanized IgG1. (Removed from the Canadian market).

Anti FcRn [72]

Definition of the therapeutic target

We have already talked about it on pages 48 . The membrane FcRn receptor is a heterodimer with a structure similar to that of the class I major histocompatibility complex molecules: an α chain and a ß2 microglobulin molecule. Present in endothelial and hematological cells, this receptor is responsible for the recycling and therefore the long t1/2 of certain IgG isotypes, particularly IgG1, IgG2, IgG4 but also albumin. It is therefore responsible for the long t1/2 of circulating IgG autoantibodies associated with the autoimmune aspect of myastenia gravis, a debilitating allo or autoimmune condition.

The monoclonal antibody directed against the α chain of the FcRn receptor is used for the treatment of myasthenia gravis. It blocks access to autoantibodies, reduces their recycling and therefore their t1/2 and their pathogenic role. This reduction in the binding of auto-Ab to FcRn is responsible for their increased lysosomal metabolism.

Monoclonal antibody

Rozanolixizumab noli (Rystiggo, UCB), humanized chimeric IgG4P, a proline (P) replaces the serine at position 241 This mutation is intended to suppress arm exchange.

Anti VLA-4 [73, 74,75, 76]

Definition of the therapeutic target

Very late integrin 4 (VLA-4) is an integrin. Its dimeric structure (α4ß1) is composed of CD49d (α4) and CD29 (ß1).

It is present on the surface of different cells of the myelocyte and lymphocyte lineages including T and B lymphocytes but also NK cells.

This integrin binds to VCAM-1 (vascular cell adhesion molecule 1, CD106) overexpressed at the endothelial level under the action of various pro-inflammatory cytokines. VLA-4 is necessary for the diapedesis of activated leukocytes towards the inflammatory site but also for the adhesion of myeloma cells in the stroma of the bone marrow.

The monoclonal antibody against CD49d is used in the treatment of multiple sclerosis. It prevents α4ß1 integrin adhesion to different proteins including CD106 of the endothelial cells limiting T-cell diapedesis across the blood-brain barrier to the inflammatory site.

The VLA-4 inhibitor has also been proposed for the treatment of Crohn's disease.

Monoclonal antibody

- Natalizumab (Tysabri, Biogen Idec), humanized IgG4.

Anti α4β7 [77, 78, 79]

Definition of the therapeutic target

Lymphocyte Peyer's patch adhesin molecule 1 (LPAM) or α4ß7 which is close to VLA-4 is one of the many integrins expressed on the surface of T and B lymphocytes in the intestinal mucosa. It mediates their migration and their residence in the lymphoid tissue associated with the intestine, Peyer's patches and the intestinal lamina propria.

By its transmembrane subunits, α4ß7 binds to the MadCAM-1 (mucosal addressing cell adhesion molecule) and VCAM expressed on the endothelial surface of the venules of the gastrointestinal tract.

The monoclonal antibody blocks the interaction of α4ß7 with MadCAM1, inhibits the migration of lymphocytes to the intestinal mucosa in Crohn's disease and ulcerative colitis.

Monoclonal antibody

- Vedolizumab (Entyvio, Takeda Pharm.), humanized IgG1.

  Unlike Natalizumab directed against α4 common to α4ß1 and α4ß7, vedolizumab binds to a structural epitope unique to the α4ß7 complex.

Anti IgE [80, 81, 82]

Definition of the therapeutic target

IgE are the Ig with the lowest plasma concentration of all isotypes. The constant part of the H chains includes 4 domains (Cε1-Cε4).

They are synthesized by plasma cells in the lymph nodes and the mucous membranes of the respiratory tract. This synthesis is under the close control of Th2 lymphocytes and various cytokines, mainly IL-4 and 13.

IL-4 stimulates the maturation of B lymphocytes into plasma cells synthesizing IgE and then into memory cells synthesizing increased amounts of IgE directed against the same antigen.

The abnormally high production of IgE in response to an allergen (antigen or hapten linked to a protein) therefore indicates an allergic condition known as atopic.

IgE exert their activity by binding, through their Cε3, to two types of receptors, one is of high (FcεRI) and the other of low (FcεRII/CD3) affinity, their structure is known. They are present on the surface of various immuno-inflammatory cells including polymorphonuclear basophils, eosinophils and mast cells. Epithelial and smooth muscle cells also express high affinity receptors at the membrane level.

The atopic allergic reaction occurs in two stages. A first step consists of sensitization during which IgE binds through its Fc fragment to the FcεRI receptors of mast cells. A second contact with the same multivalent antigen results in the bridging of 2 IgE molecules on the surface of the mast cells leading to the release of different preformed inflammatory mediators including histamine, the neosynthesis of autacoids including leukotrienes but a number of cytokines contributing to the exacerbation of the atopic asthmatic reaction.

A monoclonal antibody binds to 2 C3ε domains of circulating IgE, preventing their binding to their cellular receptors, thereby inhibiting the degranulation of mast cells and basophils and thus preventing asthma attacks and chronic atopic idiopathic urticaria.

Monoclonal antibody

• Omalizumab (Xolair, Novartis), humanized IgG1. A combination of 2 mutations (SELF: S267E/L328F) significantly increases the affinity for FCγRIIB receptors and decreases binding to FcγRIIIA receptors.

Anti C5 [83, 84, 85, 86, 87]

Definition of the therapeutic target

We saw on page 7 that complement factor C5 is, with factors C3 and C4, one of the proteins of the effector metabolic pathway onto which the three activation pathways of this system lead.

The activation of the C3 factor by a C3 convetase generates C3a and C3b. C3b, an opsonin, is also a C5 convertase; it binds to the surface of the cell to be lysed, leading to the hydrolysis of factors C4 and C5 into metabolites with inflammatory properties, including factor C5b. The latter binds to C6 and C7 to form with the factors C8 and C9 the membrane attack complex (MAC) responsible for cell lysis including hemolysis,

The enzymatic activity of C5b is controlled by 2 GPI type I membrane proteins: one, CD55 or complement decay accelerating factor (DAF) and the other, CD59, both present on the surface of different cells including endoyhelial cells and RBCs.

CD55 and CD59 limit the activity of MAC, however acting at two different levels. CD55 (DAF) limits its formation by inhibiting the activity of C3 and C5 convertases. On the other hand, C59 limits the polymerization of C9 in the formation of MAC and therefore its stability.

A genetic deficiency of CD55 leads to uncontrolled C3 convertase activity, characteristic of Chapel disease.an autosomal recessive hereditary disease.

On the other hand, an acquired abnormality of the GPI bridge of CD55 and CD59 is responsible for paroxysmal hemoglobinuria.

These different genetic conditions and others for which the use of an anti-C5 monoclonal antibody is recommended are characterized by uncontrolled MAC activity responsible, among other things, for hemolysis and hemoglobinuria.

Monoclonal antibodies

- Eculizumab (Soliris, Alexion Pharma), humanized IgG2.
- Ravulizumab (Ultomiris, Alexion Pharma), humanized IgG2 / 4.
- Pozelimab bbfg (Veopoz,Regeneron pharma), human IgG4.

Anti C1s [88]

Definition of the therapeutic target

The C1s protein is one of the 3 constituents with C1r and C1q forming the complement factor C1.

The interaction of this factor with pentameric IgM leads to activation of the classical complement pathway. It is this activation mechanism that underlies the pathophysiology of the disease associated with primary cold agglutinins but most often secondary associated with lymphoproliferative syndrome.

Indeed, the circulating blood of these patients contains cold agglutinins or cryoglobulins ie most often autoantibodies of the IgM isotype capable of forming immune complexes with carbohydrate antigens of red blood cells at temperature below 30 oC . These immune complexes bind to the complement C1 complex with triggering of the classical pathway and formation, among other things, of the opsonin C3b responsible for hemolysis.

The monoclonal antibody blocks the activation of the classical Complement pathway by the cryoglobulins.

Monoclonal antibody

- Sutimlimab (Enjaymo, Sanofi), human IgG4.

Anti P selectin [89, 90]

Definition of the therapeutic target

Sickle cell anemia is a type of anemia characterized by sickle-shaped red blood cells. This anomaly is due to the presence of hemoglobin S. This abnormal hemoglobin results from a mutation in the gene coding for its ß chain.

This hemoglobin S, in deoxygenated form, precipitates and is responsible for various complications including a vaso-occlusion resulting in a tissue infarction associated with pain and a lack of tissue oxygen supply.

This vaso-occlusion is mediated by the interaction between endothelial and platelet P selectin, a single-cell transmembrane glycoprotein, member of cell adhesion molecules (CAM), and its leukocyte ligand (P selectin ligand-1, PSGL-1).

The monoclonal antibody is directed against endothelial and platelet P selectin preventing its interaction with leukocytes and therefore preventing vasoocclusion.

Monoclonal antibody

- Crizanlizumab (SEG101, Novartis), humanized IgG2.

A word from chemokines...

Difficult to keep it simple when it's complicated!

We have already talked about chemokines. They are cytokines with a molecular mass of around 10kDa.

Their molecular structure, based on two cysteine residues in the N terminal position, allows them to be classified into 4 families.

At present, 47 chemokines have been listed, they act via 23 receptors. Some chemokines can be agonists for some receptors and antagonists for others. In addition, several chemokines can stimulate the same receptor and several receptors can be stimulated by the same chemokine.

Chemokines, according to their pharmacological properties, can be classified into two main groups: inflammatory chemokines on the one hand and homeostatic chemokines on the other. The former regulates cell movements during the development of the two types of immune reaction. The second regulates lymphocyte interactions outside inflammatory conditions ... to be continued.

Anti CCR4 [91, 92]

Definition of the therapeutic target

CCR4 or CC chemokine receptor 4 is as its name suggests a receptor with 7 transmembrane passages coupled to protein G which mediates the effects of the chemokines CCL17 and CCL22. These chemotactic cytokines are produced by cells as different as endothelial, epithelial cells, fibroblasts and macrophages stimulated by a DAMP or a PAMP or by inflammatory interleukins such as IL-1ß or TNFα. They play an essential role in the recruitment, migration and homing of cells in inflammatory, angiogenic and neoplastic processes

CCR4 is expressed mainly by $CD4^+$ Th2 lymphocytes but also Th17 and NK cells. In different types of lymphomas, it is expressed on tumor cells but also by Treg lymphocytes which inhibit the anti-tumor immune reaction.

The anti CCR4 monoclonal antibody is useful in the treatment of leukemia and T-cell lymphomas and in certain forms of cutaneous T-cell lymphomas. It antagonizes the effect of chemokines on their receptor expressed by tumor cells but also by Treg lymphocytes which inhibits the anti-tumor immune reaction.

Monoclonal antibody

- Mogamulizumab (Poteligeo, Kyowa, Amgen), Humanized type II IgG1.

Mogalizumab: A Type II IgG1.

What to say?

Type II IgG1 have appeared recently in the literature as opposed to type I IgG1.

In fact, various studies, not only clinical but also fundamental, have shown that the effectiveness of ADCC of Natural killer cells mediated by a specific IgG1 depends closely on the affinity of the Fc fragment of this IgG1 for its receptor FcγRIIIA, the only IgG1 receptor on the surface of these NK cells. This affinity itself depends, and in particular, on the nature of an oligosaccharide chain grafted onto the residue Asn 297 of the CH2 domain of this type I IgG1. Thus, the presence of a fucose residue (6-deoxy glucose of configuration L) on this N chain glycan decreases from 10 to 100 times the affinity of IgG1 for its receptor FcγRIIIA and therefore the efficiency of the ADCC of NK cells in the elimination of cells whose epitopes are linked to the paratopes of this IgG1.

These observations led to a field of new investigations and the development of type II IgG1, deprived of fucosyl residue at the level of the poly glycosidic chain in position Asn 297. The Fc fragment of these type II antibodies has an increased affinity for the Fcγ RIIIA receptors of NK cells, responsible for a more efficient ADCC. Another strategy consists of removing this glycosylation of the Fc fragment by replacing Asn 297 with Ala or by introducing other sugars such as galactose for example.

This area of research devoted to the glycosylation of the Fc fragment aimed at improving the therapeutic efficacy of monoclonal antibodies is a new field of bioengineering.

For additional useful information: Abdeldaim DT and Schindowski K. Pharmaceutics. 2023;15:2402.doi: 10.3390/pharmaceutics15102402.

Anti Interferon gamma [93, 94, 95]

Definition of the therapeutic target

Interferon gamma (IFN γ) is a peptide of 143 amino acids, the only member of type II IFN. It is secreted by NK cells, $CD4^+$Th1 and $CD8^+$ lymphocytes.

Pro inflammatory cytokine, it is the main activator of macrophages. It is also endowed with antiviral, antiproliferative and immunomodulatory properties on various cells of the immune reactions, properties mediated by a receptor made up of two peptide chains, respectively IFNγ R1 and R2.

With IL-12, it stimulates the differentiation of $CD4^+$lymphocytes into Th1 and inhibits the formation of Th2 and Th17 lymphocytes.

The monoclonal antibody is used in patients with primary hemophagocytic lymphocytosis also called macrophagic activation syndrome in which the concentrations of IFNγ are high.

Monoclonal antibody

- Emapalumab (Gamifant, Novimmune and Swedish Orphan Biovitrum), human IgG1.

Anti Interferon alpha [96, 97, 98]

Definition of the therapeutic target

Interferon alpha (IFNα) is a member of the 13 type I IFN family.

IFNα activates its receptors IFNAR1 and INFAR2 responsible for the phosphorylation of the tyrosine residues of STAT1 and 2 which leads to the translocation of IFN regulatory factor 9 to the nucleus followed by the synthesis of proinflammatory and immunomodulatory proteins involved in the host response to viral infection and amplification of type I IFN secretion

The monoclonal antibody binds to IFNAR1 inhibiting IFNα binding. It is used in the treatment of systemic lupus erythematosus.

Monoclonal antibody

- Anifrolumab (Saphnelo, Medimmune Astra Zeneca), humanized IgG1.

  This Mab contains 3 mutations in the heavy chain: L234F/ L235E/ P331S. These modifications reduce the interaction with FcγR thereby reducing ADCC and CDC.

4. Target: The Tumor (xxTUxxMAB)

Effectiveness of anti-tumor monoclonal antibodies

The primary purpose of cancer immunotherapy is to specifically kill cancer cells while safeguarding healthy cells.

The monoclonal antibodies used to kill tumor cells can act by a direct and / or indirect mechanism.

-Directly, these antibodies, by the reaction of their paratope with an epitope, inhibit or antagonize a cytokine or a growth factor interrupting intracellular signaling.

- Indirectly, the monoclonal antibody interacting with an epitope (e.g. a CD) of the target cell triggers by its Fcγ fragment CDC, ADCP or ADCC.

However, the latter mechanism (ADCC) mediated by NK cells seems to be the most important for various reasons:

-In vivo, tumor cells express CD59, CD55 and CD46 which limit MAC formation and cell lysis during activation of the complement.

-Even if the receptors Fcγ (FcγRI (CD 64), FcγRIIA (CD32a), FcγRIIB (CD 32b), FcγRIIC (CD32c), FcγRIIIA (CD16a) and FcγRIIIB (CD16b) are present on several types of cells (NK cells, monocytes, macrophages, neutrophils, eosinophils and dendritic cells), the cytotoxicity of a monoclonal IgG depends on the difference in its affinities for an activating receptor and the inhibiting receptor FcγRIIB.

Thus, the role and the number of NK cells seem decisive in cancer immunotherapy. Numerous studies show that NK cells are the main cytotoxic cells in vivo. Indeed, NK cells do not have the FcγRIIB inhibitor receptor, but only the FcγRIIIA receptor, activator of ADCC.

The importance of the IgG1… type II isoform should also not be overlooked.

Anti CD3 [99, 100, 101, 102]

Definition of the therapeutic target

CD3 is a transmembrane protein complex common to T cells. It consists of 4 polypeptide chains: a CD3γ chain, a CD3δ chain and two CD3ε chains.

These transmembrane proteins associate with the T-cell receptor (TCR) and a ξ chain to form the TCR complex, the central part of the immune synapse, and generate the intracellular signals responsible for T cell activation. Thus, CD3 plays a key role in the activation of CD4+ and CD8+ T lymphocytes in contact with a APC-peptide-MCH I or MCHII complex.

The first anti CD3 monoclonal antibody (OKT3) directed against the ε chain of the CD3-TCR complex has been successfully used to prevent transplant rejection of various organs. However, this mouse monoclonal antibody is immunogenic in human. This immunogenicity is responsible for severe side effects. To overcome the production of HAMA, other anti CD3 monoclonal antibodies have been developed, they are described below.

Monoclonal antibody

- Muromomab (OKT3, Janssen), mouse IgG2a.
- Teplizumab (Tzield, Lilly), humanized IgG1 with a modified Fc fragment (LALA mutation: Leu234Ala and Leu235Ala) to decrease its interaction with Fcγ receptors.

  It is used to delay the onset of stage 3 of type 1 diabetes mediated by T cells with destruction of B cells by autoactivation CD4+ and CD8+ T lymphocytes.

Anti EpCAM and anti CD3 [103]

Definition of the therapeutic target

Epithelial cell adhesion molecule (EpCam), also known as CD326, is a transmembrane glycoprotein expressed solely on epithelial cells and epithelial tumor cells. It is essestial for the differenciation, the proliferation and adhesion of these normal and pathological cells.

EpCAM is a marker of differentiation of cancers with poor prognosis.

The monoclonal antibody is used intra peritoneally in patients with severe ascites associated with epithelial carcinoma (ovarian, intestine, stomach ...) to destroy epithelial cells with EpCAM antigen, these cells being the cause of ascites.The peritoneum being of mesothelial origin, it does not express epCAM.

Monoclonal antibody

- Catrumaxomab (Removab, Frezenius Biotech, TRiOn Pharma), IgG2 hybrid mouse-rat. The mouse IgG2a fragment binds to CD3 on lymphocytes while rat IgG2b binds to EpCAM on the tumor cell.

Anti CD3 and anti GPRC5D [104]

Therapeutic target

CD3 has already been defined. GPRC5D (G protein coupled receptor class C group 5 member D) is an orphan receptor encoded by the eponymous gene poorly expressed in different types of normal cells but overexpressed on malignant plasma cells.

The MAB is used in the treatment of multiple myeloma.

Monoclonal antibody

Talquetamab tdvs (Talvey, Janssen Pharmaceutica) bispecific IgG4λ with a PAA mutation (S228P/L234A/L235A) to prevent arm exchange.

Anti CD19 [105], [106], [107]

Definition of the therapeutic target

CD-19 is a membrane marker of B lymphocytes. Transmembrane glycoprotein (95KDa), it belongs to the Ig superfamily. CD19 is associated with CD21 and CD81, forming the B cell co-receptor. Activation of this receptor leads to phosphorylation of cytoplasmic tyrosine residues.

CD19 is expressed at all stages of development of normal and neoplastic B lymphocytes to plasma cells. It is essential for the development of B lymphocytes and their activation when Ag comes into contact with BCR.

It is also present on the membrane of follicular dendritic cells.

CD19 is present on the cells of patients with non-Hodgkin's lymphoma (NHL,) acute lymphoblastic leukemia (ALL) or chronic lymphocytic leukemia. Unlike CD20, it is more uniformly expressed on the cell membrane. CD19 is also present on CD20 negative cells.

Monoclonal antibodies

- Loncastuximab Tesirine (Zynlonta ADC therapeutics), humanized IgG1 is an ADC (antibody drug complex).

  The toxin, an alkylating agent, binds to DNA, interferes in its replication and blocks cell division. Used for the treatment of DLBCL (Diffuse large B cell lymphoma).

- Tafasitamab (Incyte, Morphosys US Inc), humanized IgG1/IgG2 heterodimer for the CH2 and CH3 domains. The hinge region and the Fab are IgG1 .

  The Fc receptor has been modified by substituting 2 AA: S239D and I332E to increase the affinity with the Fcγ RIIIA receptors and ADCC.

  Lenalidomide is an analogue of talidomide which inhibits tumor angiogenesis and the secretion of interleukins by the tumour. It Leads to apoptosis and inhibition of cell proliferation.

- Inebilizumab (Uplizna, Medimmune), humanized IgG1 whose Fc fragment has been modified (fucose free, type II IgG1) to increase the binding to the Fcgγ RIIIA leading to the activation of NK cells.

  This monoclonal antibody was developed for the treatment of neuromyelitis optica seropositive for IgG autoantibodies against aquaporin 4 (AQP4). These autoantibodies activate the C responsible for astrocyte damage and an inflammatory reaction.

Anti CD19 and anti CD3 [108, 109, 110]

Definition of the therapeutic target

CD3 and CD19 have been defined.

Monoclonal antibody

- Blinatumomab (Blincyto, Amgen), BiTE of murine nature.

  The monoclonal antibody is a BiTE (Bispecific T lymphocyte Engager). Single-strand fusion protein (54,1 kDa), it has two Fab sites. One binds to CD19 on B cells, the other to CD3 of cytotoxic T lymphocytes allowing the destruction of B lymphocytes. This BiTE has some efficacy in the treatment of acute lymphoid leukemia.

Anti CD3 and anti BCMA [111, 112, 113, 114]

Definition of the therapeutic target

BCMA (B cell maturation antigen) is a transmembrane glycoprotein, member of the super family of TNF receptors (TNFRSF 17) which recognizes the B cell activating factor (BAFF) counterpart of TNFα.

Binding of BAFF to BCMA leads to activation of MAPK 8/JNK and NFkB responsible for cell proliferation.

BCMA is expressed on the membrane of myeloma cells and some healthy B lymphocytes. BCMA is found in soluble form in circulating blood at high concentration in multiple myeloma

The monoclonal antibody is used in the treatment of relapsed or refractory multiple myeloma.

Monoclonal antibodies

- Teclistamab (Tecvayli, Jansen, Johnson and Johnson), humanized IgG4 is a fusion bispecific protein (BiTE).

Elranatamab bcmm (Elrexflo, Pfizer),bispecific humanized IgG2.

Anti CD3 and anti GP100 [115, 116, 117]

Definition of the therapeutic target

Gp100 or PEML melanoprotein is a transmembrane glycoprotein of melanosomes that produce melanin in melanocytes.

Monoclonal antibody

- Febentafusp (Kimmtrack, Immunocore) is a bispecific fusion protein (BiTE) used in the treatment of uveal melanoma.

Anti CD20 [118, 119, 120]

Definition of the therapeutic target

The already defined CD20 is overexpressed on the surface of non-Hodgkin's lymphoma (NHL) cells that originate from B-cells.

Monoclonal antibodies are used for first-line treatment but also for refractory $CD20^+$ NHL. The binding of a monoclonal antibody to CD20 interrupts intracellular transduction signals.

Monoclonal antibodies

- Rituximab (Rituxan, Biogen Idec, Gennentech), chimeric IgG1,
- Ibritumomab (Zevalin, Biogen Idec), chimeric IgG1 labeled with ^{90}Yttrim, a ß emitter. The structure of this antibody is as follows: IgG1-tiuxetan-^{90}Yttrium.

  After binding to CD20, the chelator (tiuxetan) is hydrolyzed and the released ^{90}Yttrium is internalized.
- Ofutumumab (Arzerra, Genmab and GlaxoSmithKline), type II human IgG1.
- Obinutuzumab (Gazyva, Roche), type II humanized IgG1.
- Tositumomab (Bexxar, GlaxoSmithKline), IgG2 λ labeled with ^{131}I.

Anti CD3 and anti CD20 [121, 122, 123]

Therapeutic targets

Have already been defined.

Monoclonal antibodies

- Mosunetuzumab (Rylase, Lunsumio, Roche, Genentech), humanized IgG1, a BiTE for the treatment of non-Hodgkin's lymphoma.
- Epcoritamab bysp (Epkinly, Genmab, Abbvie), bispecific humanized IgG1 κ/λ to treat refractory large B-cell lymphomas

Glofitamab gxbm (Columvi, Hoffman-Laroche) bispecific IgG1 κ/λ anti CD20 et CD3ε. The Fc fragment has a P239G-LALA mutation, resulting in the loss of binding to the FcγRIII receptors without affecting its stability or its binding to the FcRn receptor, responsible for its long t1/2.

Anti CD22 [124]

Definition of the therapeutic target

CD22 is a membrane sialoglycoprotein, a lectin member of the SIGLEC (sialic acid-binding immunoglobulins-type lectins). It is expressed on B cells and their precursors, but not plasma cells. During ontogenesis, it appears before CD20 and accompanies the expression of IgD and IgM. Due to its tyrosine phosphatase properties, it has a suppressor role during the BCR activation by the Ag.

It is overexpressed in more than 90% of malign B lymphocytes in patients with acute lymphoblastic leukemia, chronic lymphocytic leukemia and NHL.

The monoclonal antibody coupled to a cytotoxic antibiotic, ozogamicin, binds to CD22. This complex is internalized before being hydrolysed by lysosomal enzymes. The released cytotoxin denatures DNA leading to apoptosis.

Monoclonal antibody

- Inotuzumab ozogamicine (Besponsa, Pfizer), humanized IgG4 is an ADC.

Anti CD30 [125, 126]

Definition of the Therapeutic target

CD30 also known as TNFRSF8 is a transmembrane receptor, member of the TNF receptor family. It is poorly expressed in normal tissues but overexpressed in different lymphocytic neoplasms.

Monoclonal antibody is effective in different primary effusion lymphoma (PEL).

Monoclonal antibody

- Brentuximab-vedotin (Adcetris, Seattle Gen), chimeric IgG1, an ADC. This antibody is conjugated to four molecules of the antineoplastic agent (monomethyl auristatin E)

  It causes apoptotic death of cells expressing CD30. The CD30-Brentuximab complex is internalized by endocytosis, the ligand is digested in the lysosomes. The antineoplastic agent is released, and it binds to the microtubule network, leading to the interruption of the cell cycle. Thus, brentuximab-vedotin is a microtubule disrupting agent.

Anti CD33 [127]

Definition of the therapeutic target

CD33 or SIGLEC-3 (Sialic acid binding Ig-like lectin 3) is, by its structure, a member of the Ig superfamily. It is expressed, inter alia, on the surface of the precursor cells of the myelocyte line. CD33 is overexpressed in 80% of patients with acute myeloid leukemia, hence the clinical relevance of anti-CD33 for the treatment of acute myeloid leukemia.

Monoclonal antibody

- Tuzumab (Mylotarg, Pfizer), humanized IgG4 coupled with cytotoxic ozogamicin. (see CD22), an ADC.

Anti CD38 [128, 129, 130]

Definition of theTherapeutic target

CD 38 is a multifunctional membrane ectoenzyme that catalyses the synthesis and the hydrolysis of cyclic adénosine-5-diphosphoribose (cADPR), involved in the regulation of metabolism and functions of intracellular Ca^{++}.

CD38 is weakly expressed on the cell surface of lymphocyte and myelocyte lines but overexpressed in multiple myeloma cells. In chronic lymphocytic leukemia, this overexpression makes it an unfavorable marker for the progression of the disease.

The monoclonal antibody anti CD38 is used in the treatment of multiple myeloma.

Monoclonal antibodies

- Daratumumab (Darzalex, Janssen), humanized IgG1.
- Isatuximab (SAR-650094, Immunogen, Sanofi Aventis), humanized IgG1.

Anti CD52 [131, 132]

Definition of the Therapeutic target

A sequence of 12 amino acids attached to the cell membrane by a GPI bridge, the CAMPATH1 antigen (Cambridge pathology I) is a glycoprotein of unknown function. It is expressed on more than 95% of circulating lymphocytes, mainly T lymphocytes.

Its overexpression is associated with certain types of lymphoma.

Anti CD52 is used for the treatment of chronic B lymphocytic leukemia.

Monoclonal antibody

- Alemtuzumab (Lemtrada, Sanofi), humanized IgG1.

Anti tissue factor [133, 134]

Definition of the therapeutic target

Tissue factor (TF, CD142, factor III or platelet tisse factor) is a transmembrane glycoprotein.

TF is present on the membranes of endothelial cells and leukocytes. It is also expressed in fibroblasts and subendothelial smooth muscle cells. Endowed with serine protease activity, it activates coagulation factor VII leading to activation of the intrinsic pathway via factor X and thrombin. TF also plays a role in tumor-associated angiogenesis, the spread of metastases. TF is overexpressed in different solid tumors. The expression of TF correlates with the presence of metastases, angiogenesis and thrombosis associated with tumors including cervical cancer and certain cancers with poor prognosis.

The monoclonal antibody is used for the treatment of recurrent or metastatic cervical cancer

Monoclonal antibody

• Tisotumab-vedotin (Tivdak, Genmab) Human IgG1. Binding of the ADC antibody to TF is followed by internalization of vedotin which inhibits tubulin polymerization with arrest of the G2/M phase leading to apoptosis.

Anti folate receptor [135, 136]

Definition of the therapeutic target

Folate or more particularly vitamin B9 is necessary or even essential for cellular metabolism, DNA synthesis and metabolism.

Vitamin B9 and folate in general are transported inside the cell by endocytosis via an FR receptor, 4 in number: FRα, ß, γ and δ.

FRα, also known as FOLR1, is a membrane GPI protein little or not expressed in normal tissues but overexpressed in epithelial cancers, including 80% of ovarian carcinomas, giving malignant cells a growth advantage. FOLR1 overexpression correlates with histological examination and the stage of the solid tumor.

The monoclonal antibody has been approved for the treatment of ovarian, fallopian tube and primary peritoneal cancer.

Monoclonal antibody

- Mirvetuximab (Elahere, Immunogene), humanized IgG1 coupled to soravtansine which inhibits cell division, an ADC.

Anti EGF Receptor (EGF-R) [137], [138]

Definition of the therapeutic target

EGF, but also TGFα, exerts its physiological activity by binding to a transmembrane receptor (EGF-R or ErbB-1) of tyrosine kinase type responsible, after dimerisation, for the transduction of intracellular signals and cell proliferation.

EGF does not act only at the level of the epidermis. Its receptor, involved in tissue development and healing, is overexpressed in many tumors including non-small cell lung cancer. Its expression is a bad prognosis.

The monoclonal antibodies are directed against the extracellular portion of EGF-R, preventing its dimerization and activation. They cause its internalization and its degradation followed by the death of the tumor cell by ADCC. The presence of EGF-R in the epidermis explains the side effects of these monoclonal antibodies at this level.

Monoclonal antibodies

- Cetuximab (Erbitux, Eli Lilly, Merck Serono and Bristol Myers Squibb), chimeric IgG1.
- Necitumumab (Portazza, Eli Lilly), humanized IgG1.
- Panitumumab (Vectibix, Amgen), human IgG2.

Anti HERB2 [139, 140, 141, 142]

Definition of the therapeutic target

Human Epidermal growth factor 2 receptor (HERB2 or HER2 / neu or ErbB-2) is with EGF-R1 (ErbB1), HER3 (ErbB-3) and HER4 (ErbB-4) one of the four transmembrane receptors members of the ErB family, with cytoplasmic tyrosine kinase activity.

HERB2 is activated by homodimerization but also by heterodimerization with another ErbB, its ligand remains unknown.

Involved in normal breast development, it is overexpressed in 20-30% of breast cancers, but also other tumors. This overexpression is associated with mutations leading to cell proliferation insensitive to hormone and chemotherapy.

Monoclonal antibodies bind to the extracellular portion of the HERB2 receptor preventing dimerization followed by internalization and cell death.

Monoclonal antibodies

- Trastuzumab (Kadcyla, Roche), humanized IgG1.

  Biosimilar: Ogivri (Mylan GMBH), humanized IgG1.

- Pertuzumab (Perjeta, Roche), IgG1 developed to overcome resistance phenomena observed with trastuzumab.

- Mergetuxumab (Mergenza Macrogenics), chimeric IgG1 is a structural modification of trastuzumab. Its paratope is identical and therefore has an identical specificity to that of trastuzumab. Its Fc fragment has been modified at the level of 5 amino acids to increase its affinity for the CD16a receptor (FcγRIIIA) and decrease its affinity for CD32b (FcγRIIB), an inhibitory receptor. This modification leads to an increase in ADCC via CD16a,

Anti TROP2 [143, 144, 145]

Definition of the therapeutic target

Trophoblast antigen 2 (TROP 2) is a transmembrane calcium signal transducer weakly expressed in the epithelial cells of normal tissues but overexpressed in various types of cancers including breast cancer in which it plays the role of an oncogene contributing to tumor proliferation.

The anti TROP 2 antibody is currently used in triple negative breast cancers for estrogen, progesterone and HERB receptors, with poor prognosis.

Monoclonal antibody

- Sacituzumab- govitecan (Trodelvy, Immunomedic), humanized IgG1, an ADC. Govitecan is a topoisomerase inhibitor.

Anti PDGF receptor (PDGFR) [146, 147, 148]

Definition of the therapeutic target

The Platelet Derivative Growth Factor (PDGF) receptor, a transmembrane glycoprotein with tyrosine kinase activity, exists in two forms: α and ß which dimerize (αα, ßß and αß) when subjected to one of their ligands, one of the five PDGFs, homo but also heterodimers (AA, BB,CC, DD and AB).

As the name suggests, PDGF is mainly but not exclusively synthesized and secreted by platelets.

It is endowed with angiogenic and mitogenic properties, stimulating the proliferation of cells of mesothelial origin.

The monoclonal antibody antagonizes PDGF by binding to the α-fraction of dimerized PDGFR overexpressed in some sarcomas.

Monoclonal antibody

- Olaratumab (Lartrivo, Eli Lilly), human IgG1.

Anti SLAMF7 [149]

Definition and therapeutic target

Self ligand receptor of the signaling lymphocytic activation family, SLAM F7 is a member of the SLAM family also called CS1 or CD319. The SLAM family belongs to the receptors of the Ig super family.

The glycoprotein SLAMF7 is expressed on NK cells, T lymphocytes, monocytes, B cells and many tissue cells. It plays an important role in immunoregulation. This receptor is universally expressed on the surface of multiple myeloma cells.

Mab binds to SLAMF7. Once bound, the monoclonal antibody recruits NK cells via its FcγIII receptors.

Monoclonal antibody

- Elotuzumab (Emphiciti, Bristol Myers Squibb), humanized IgG1.

Anti GD2 [150, 151]

Definition of the therapeutic target

GD2 is a ganglioside expressed on the surface of different stem cells before birth but also on the surface of neuronal cells and melanocytes.

Its role is poorly known. However, like other membrane gangliosides, it functions as a receptor for microbial toxins and as an adhesion molecule.

The clinical interest of GD2 lies in its overexpression in neuroblastoma, but not exclusively.

Monoclonal antibody

- Dinutuximab (Unituxin, Eusa Pharma), chimeric IgG1.
- Naxitamab (Denyelza, Y mabs therapeutics),humanized IgG1, in combination with GM-CSF for the treatment of neuroblastoma.

Anti CD79b [152]

Definition of the therapeutic target

CD 79b (Igß) forms with CD79a (Igα) the BCR (IgM or IgD) co-receptor on the membrane of B lymphocytes. The heterodimer CD79a / CD79b is responsible for the transduction of the intracellular signal which leads to the activation, proliferation and differentiation of B lymphocytes.

CD79b expression is restricted to mature B cells and non-Hodgkin's lymphoma (NHL) B cells.

The monoclonal antibody is used in the treatment of relapsed or refractory diffuse large B cell lymphoma.

Monoclonal antibody

- Polatuzumab-vedotin (Poliny, Genentech Roche), humanized IgG1 coupled to a cytotoxic agent.

  Like brentuximab vedotin, polatuzumab vedotin is an ADC. After binding to CD79b on the surface of the B lymphocyte, this ADC complex is internalized; after fusion with a lysosome, the arm is hydrolyzed and vedotin inhibits cell division which leads to apoptosis.

Anti IGF-1R [153, 154]

Definition of the therapeutic target

IGF-1R is one of the 2 IGF-1 receptors for Insulin growth factor 1 also called somatomedin C.

IGF-1R, a tyrosine kinase receptor, consists of 2 α subunits and two ß subunits. It has a structural analogy with the insulin receptor. It is overexpressed by orbital fibroblasts in ocular orbitopathy associated with Graves' disease also called thyroid eye disease, an autoimmune disease characterized by the proliferation of orbital fibroblasts and infiltration of the eye socket by lymphocytes sources of cytokines such as IL -1ß, IL-6. IL-16 or TNFα which induce the synthesis of glycosaminoglycans and the characteristic adipogenesis and inflammation of the orbit.

This periorbital inflammation is initiated by two types of autoantibodies. On the one hand, those that target the TSH receptor and on the other hand, anti-IGF-1R autoantibodies. The synergy of these two receptors is involved in the pathogenesis of autoimmune orbitopathy.

The monoclonal antibody binds to the α extracellular domain of IGF-1R and blocks the activation of IGF-1R by the corresponding autoantibodies

Monoclonal antibody

- Teprotumumab (Tepezza, Horizon Therapeutics111), human IgG1 .

5. Target : The toxin (xxTOXxxMAB)

Anti Clostridium difficile toxin [155, 156]

Definition of the therapeutic target

Clostridium difficile is an anaerobic gram-positive bacillus responsible for serious life-threatening nosocomial gastrointestinal infections.

Up to 35% of patients develop Clostridium difficile infection after antibiotic therapy.

Its pathogenic power is associated with two toxins: an enterotoxin (toxin A) and a cytotoxin (toxin B). The genes coding for these 2 toxins have been identified as well as the factors regulating their synthesis. The 2 toxins share a certain homology of structure and activity.

They bind to the epithelial cells of the intestinal mucosa, undergo endocytosis and result in apoptosis.

The monoclonal antibody binds and neutralizes toxin B preventing its adhesion to the cells of the intestinal mucosa.

Monoclonal antibody

- Bezlotoximab (Zinplava, Merck), humanized IgG1.

Anti Anthrax toxin [157, 158]

Definition and therapeutic target

The toxin associated with Bacillus anthracis spores is responsible for anthrax. Inhalation of the trimeric toxin associated with Bacillus anthracis spores may be used in bioterrorist attacks.

Monoclonal antibodies against the protein PA (Protective antigen) of this toxin are used in the prevention and treatment of possible bioterrorist attacks.

Monoclonal antibodies

- Raxibacumab (Abthrax, GlaxoSmithKline), human IgG1λ.
- Obiltoxaximab (Anthim, Elusys Therapeutics), chimeric IgG1.

6. Target: the virus (xxVIxxMAB) [159, 160, 161, 162]

Anti syncitial virus

Definition of the therapeutic target

Syncitial virus (RSV) is a single-stranded RNA virus, it belongs to the group of pneumoviruses. It is the main cause of severe respiratory infections and in particular bronchiolitis in newborns and infants.

The monoclonal antibody is directed against an epitope of the F protein of the RSV, it is used in newborns and high-risk infants.

Monoclonal antibodies

- Pavilizumab (Synagis, Medimmune Abott), humanized IgG1.
- Nirsevimab (Beyfortus, Astra Zeneca), IgG1κ binds to the F1 and F2 subunits of the RSV fusion protein blocking the entry of the virus into the cell.

  The Fc fragment was modified (M252Y, S254T , T256E) to increase in vivo t1/2 from 21-28 days to 87-117 days. An intramuscular dose results in 5 months protection.

Anti-SARS-CoV-2 [163, 164, 165, 166, 167, 168, 169]

We summarize here in a schematic way, perhaps too schematically, the current knowledge relating to this virus relevant for immunotherapy. The interested reader will find additional relevant literature in the references mentioned below.

Definition of the therapeutic target

SARS-CoV-2 (Severe acute respiratory syndrome coronavirus-2), responsible for Covid 19 (coronavirus disease 2019) is a single-stranded RNAm virus with positive polarity. Along with MERS-CoV (middle east respiratory syndrome) and SAR-CoV, it is a member of the beta coronavirus genus.

The RNAm genome of SARS-CoV-2 has nearly 30,000 nucleotides, its replication is mediated by an RNA-dependent RNA polymerase and an exoribonuclease. This RNAm codes for around thirty proteins, the main ones at the structural level being four in number. These are respectively the S (spike), E (envelope), M (membrane) and N (nucleocapsid) proteins. The latter envelops the mRNA while the S, E and M proteins constitute the viral envelope.

So far, only the S protein is of interest for the pathogenicity and transmissibility of the virus and therefore as a target for active and passive immunotherapy. Very little or nothing is known about a potential role of other proteins in the pathogenicity of SARS-CoV-2

The S protein, also called spike, is a 1273 AA protein coated with protective polysaccharide molecules.

The pathogenicity and transmissibility of the virus are based on 3 essential structural components of this S protein: the presence of two domains respectively S1 and S2, separated by a cleavage site. The first of these domains, S1, is responsible through its RBD (Receptor bind domain, AA 319-541) for the binding of the virus to the membrane ACE2 mainly of cells of the respiratory tract. The entry of SARS-CoV-2 into airway cells is mediated by the fusion of the S2 domain with the recipient cell membrane after cleavage (FCS: furin cleavage site) of a polybasic sequence S1-S2 by a protein of the 'host, furin. This and therefore the FCS are essential for in vivo replication, transmissibility and pathogenicity of the virus.

The number and nature of mutations affecting the part of the mRNA coding for the S protein and in particular the RBD and the FCS are responsible for the outbreak of numerous variants and sub-variants, for example the Omicron variant and its sub-variants. responsible of antigenic escape. The latter renders ineffective the specificity of the Ag-Ac reaction both in vitro

and in vivo, essential to the effectiveness of active and passive immunotherapy.

Monoclonal antibodies

In 2021, the FDA granted emergency use authorization to various monoclonal antibodies for non-hospitalized patients who tested positive in the laboratory test and were at high risk of worsening and/or hospitalization. These are recombinant human IgG1 produced from a memory lymphocyte isolated from the blood of a patient who survived COVID 19. They are used two by two to avoid antigenic escape associated with a possible modification of the epitope. .

These are :

- Bambalimab and Etesevimab (Ely Lilly)
- Casirivimab and Imdevimab (Regeneron)
- Cilgavimab and Tixagevimab (AstraZeneca)
- Sotravimab (GSK , Vir Biotechnology)
- Regdanvimab (Cell trion)
- Bebtelovimab (Eli Lilly)

The FDA has also withdrawn its authorization from some of them because of antigenic escape associated with a possible modification of the epitope of the variant or sub-variant of SARS-CoV-2...to be continued.

Anti Ebola virus [170, 171]

Definition of the therapeutic target

The Ebola virus named after the river of the same name is a single-stranded RNA flavovirus of negative polarity. It is the causative agent of severe hemorrhagic fevers most often fatal.

Among the RNA-encoded proteins, the GP1.2 protein is responsible for binding the virus to the Nieman Pick C1 (NPC1) cellular receptor.

The monoclonal antibody was synthesized from a memory lymphocyte taken from a patient who survived hemorrhagic fever in 1995 in Kikwit in the DRC. This immortalized B cell was used to produce plasmid rDNA.

The monoclonal antibody binds to a peptide sequence (epitope: LEIKKPDGS) of the GP1.2 protein inhibiting its NPC1 receptor binding.

Anticorps monoclonal

- Ansuvimab (Ebanga, Medimmune), human IgG1 .

7. Target: The neural system (xxNUxxMAB)

Anti CGRP [172]

Definition of the therapeutic target

Calcitonin Gene Related Protein (CGRP) is a neuropeptide of 37 amino acids, member by its structure but not by its functions, of the family of calcitonin. As the name suggests, it results from an alternative splicing of the gene encoding calcitonin.

CGRP exists in two isoforms α and ß, which have identical biological activities, but different expressions.

Only CGRPα is present in the trigeminal system at the level of the sensory neurons of the dorsal root ganglia and in the vagal ganglia. CGRP exerts a vasodilatory and pro nociceptive action by mainly activating a multimeric receptor coupled to a G protein.

The role of CGRP in migraine results from a vasodilatory action at the level of the intracranial arteries and a modulation of neuronal excitability facilitating the painful response in the trigeminal system.

The monoclonal antibody antagonizes the binding of CGRP to its receptor. It is used prophylactically but also in the treatment of episodes of acute migraine.

Monoclonal antibodies

- Erenumab (Aimovig, Amgen), humanized IgG2λ.
- Fremanezumab (Ajovy, Teva), humanized IgG2.

Anti amyloid B [173, 174]

Definition of the therapeutic target

Amyloid ß peptide is a 36-43 AA peptide, the main component of amyloid plaque.

This peptide results from the sequential cleavage of a transmembrane protein precursor by α and ß secretases. These ß monomers aggregate in different forms and constitute the extracellular amyloid plaque which, together with the intracellular tau protein, is characteristic of Alzheimer's disease.

A monoclonal antibody has been developed to treat Alzheimer's disease. It targets ß amyloid plaques in the brain reducing their buildup.

Monoclonal antibody

- Aducanumab (Aduhelm, Biogen and Elsai), human IgG1. Approved in USA but not in Europe, neither in Canada.
- Lecanemab lrmw (Lequembi Eisai Co et Biogen Inc), humanized IgG1.

Anti-kallikrein [175]

Definition of the therapeutic target

Prekallikrein is one factor in the so-called plasma contact system. It is activated in kallikrein, a serine protease, upon contact of the plasma with a negatively charged surface. This kallikrein releases bradykinin from the high molecular weight kininogen. Bradykinin is a powerful vasodilator; it increases vascular permeability and extravasation. It is metabolized to different inactive peptides but also to another vasodilator peptide, desArg9-Bradykinin.

The enzymatic activity of plasma kallikrein is neutralized by the C1 esterase inhibitor, antiprotease, whose inherited quantitative or qualitative deficit characterizes angioedema types I and II.

The monoclonal antibody specifically inhibiting the enzymatic activity of plasma kallikrein is intended to prevent attacks of angioedema.

Monoclonal antibody

- Lanadelumab (Takhzyro, Shire), human IgG1.

8. Target: The bone (xxOSxxMAB)

Anti RANK [176, 177]

Definition of the therapeutic target

eceptor activator for nuclear factor kappa B (RANK) is a transmembrane receptor expressed by osteoclasts and their precursors.

Its ligand RANKL produced by osteoblasts is the main regulator of osteoclastic activities in bone resorption.

The binding of RANKL to RANK activates NF-κB and the expression of important genes involved in the differentiation, survival and activity of osteoclasts.

Osteoprotegerin is a natural inhibitor of RANKL, inhibiting its interaction with RANK blocking the differentiation of osteoclasts.

The expression of RANKL is modulated by various hormones and cytokines including TNFα and IL-4.

The high affinity monoclonal antibody binding to RANKL prevents its binding to RANK on the surface of osteoclasts and their precursors, decreasing the formation, survival and function of osteoclasts, thereby increasing bone density associated with a lower risk of fractures.

Monoclonal antibody

- Denosumab (Xgeva, Amgen), human IgG2.

Anti FGF23 [178, 179, 180]

Definition of the therapeutic target

The fibroblast growth factor 23 (FGF23) is, as its name suggests, a growth factor, secreted by osteocytes and osteoblasts.

At the renal tubule, FGF23 binds to a receptor member of the FGF family which forms a heterodimer with the Kloto protein. It decreases the reabsorption of phosphates, resulting in an inhibition of the secretion of PTH, the synthesis of calcitriol and an increase in its metablolism.

Serum FGF23 levels are elevated in patients with X chromosome hypophosphatemia, a form of osteomalacia associated with a mutation in the gene encoding PHEX (Phosphate-regulating endopeptidase homolog X-linked). This high concentration of FGF23 leads to renal phosphate leakage by a decrease in its tubular reabsorption responsible for hypophosphatemia.

The monoclonal antibody inhibits the activity of FGF23, increasing the tubular reabsorption of phosphate.

Monoclonal antibody

- Burosumab (Cryovita, Ultragenix and Kyowa Hakko Kirin), human IgG1.

In conclusion, in this second part, we have tried to apply to immunotherapy the knowledge of fundamental immunology previously summarized. First of all, this knowledge allows us to better understand and objectify the effectiveness of vaccination and serotherapy, long empirical. Then, using these same basic concepts, we were able to define the nature of FDA approved monoclonal antibodies, not counting biosimilar antibodies, and describe their specific therapeutic targets used for the prevention and treatment of diseases, some of which were up to there incurable. This description is succinct, perhaps; however, the references relevant to each monograph should serve as a guide to knowledge for any curious mind seeking more in-depth knowledge.

GENERAL DISCUSSION

The number of consultations and downloads of both the English and French first versions demonstrates the interest aroused by this course posted in 2023 on the papyrus site of the University of Montreal.

This interest therefore justifies the updating of an educational document dedicated to immunotherapy and particularly to monoclonal antibodies. Its scientific content, we hope, is intended to be useful, not only for specialists in immunology but also for students, health professionals and patients treated with such pharmacological agents.

Last year, in 2023, the FDA approved eleven new monoclonal antibodies for therapeutic use. Of these, only two are dedicated to a new therapeutic target. Of these new antibodies, five are bifunctional: inspiration or competition for chimeric antigen receptors (CAR)-T cells?

Surprisingly, six of these new antibodies are of the IgG4 isotype carrying one or more mutations in order to modulate their pharmacological properties and limit their undesirable effects. Could the IgG1 isotype be dethroned?

The COVID-19 epidemic has revolutionized both active and passive anti-viral immunotherapy. Anti-SARS-CoV-2 vaccination has established the effectiveness of vaccines using mRNA, the culmination of three decades of research crowned by a Nobel Prize: *Labor improbus omnia vincit*!

In 2022, the FDA granted emergency authorization to different monoclonal antibodies directed against the spike protein of the SARS-CoV-2 and prepared from memory cells of convalescent patients. The antigenic escape of certain variants and sub-variants of the virus has illustrated, if necessary, the importance of specificity, one of the two cornerstones, along with affinity, of the Antigen-Antibody reaction.

Sensitive to the comments received for the first edition, we have enhanced our text with new boxes whose content should contribute to a harmonious transfer of knowledge.

In conclusion, both in substance and form, we hope that this update contributes a little to the task assigned to us: teaching.

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