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BIOLOGICAL THERAPIES AS ALTERNATIVE TREATMENTS AGAINST ANKYLOSING SPONDYLITIS

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ABSTRACT

Ankylosing spondylitis is an autoimmune disease characterized by wear and inflammation of the spinal column, spinal cord, sacroiliac joint, and peripheral joints, affecting patients' quality of life. The treatment given against this disease involves the use of non-steroidal anti-inflammatory drugs, glucocorticoids, and antirheumatics. The objective of this therapy is to face the ailments generated by the clinical manifestations. However, thanks to the development of monoclonal antibodies, it is possible to decrease symptoms and stop the disease's progression. These drugs are therapeutic tools that act on diverse immune system targets, mainly blocking pro-inflammatory cytokines such as TNFα and IL-17a. Adalimumab, infliximab, golimumab, certolizumab pegol, secukinumab, and ixekizumab are currently commercially available. Likewise, there are many others in different investigation phases. Although there is no 100 % effective biological drug for treating ankylosing spondylitis, this scenario opens future research possibilities.

KEYWORDS: ankylosing spondylitis, immune system, cytokines, biological therapy, monoclonal antibodies, industrial production.

INTRODUCTION

Ankylosing spondylitis is an autoimmune, genetic disorder, which affects a small percentage of the world's population. Its prevalence is not well known, as it varies according to the region. Nonetheless, it is estimated between 0.03 and 1.8 % in North America, Europe, and China.[1]

A relationship has been found between this illness and the class I major histocompatibility complex (MHC) or, as known in humans, the human leukocyte antigen (HLA) because of specific polymorphisms related to the gene that encodes for HLA-B27. The data indicate that between 0.5 and 1 % of its carriers suffer from the pathology. In Costa Rica, 6 % of the population has this protein. Furthermore, other genes associated are HLA-B60 and HLA-DR1.[2, 3, 4]

This condition is mainly treated with non-steroidal anti-inflammatory drugs (NSAIDs), antirheumatic drugs, and corticosteroids. Nevertheless, they only reduce the ailments related to spondylitis and present severe adverse effects.[5]

For this reason, for more than two decades, the use of biological therapies such as monoclonal antibodies (molecules developed to restore, improve, or imitate the patient's immune response) has been implemented. These options have side effects like immunosuppression and anaphylactic shocks, but they are more directed at the disease and affect other organs to a lesser extent.[5, 6]

Against this background, this review aims to present the distinct monoclonal antibodies commercialized and studied for the ankylosing spondylitis treatment.

OVERVIEW OF ANKYLOSING SPONDYLITIS

It is an autoimmune, chronic, and inflammatory disease that reduces the quality of life of people. It is considered a progressive and debilitating immune-mediated arthropathy, challenging to diagnose.[7, 8]

The immune response to specific autoantigens in autoimmune diseases contributes to tissue damage during illness progression.[9] In this case, there are genetic factors that can trigger this response.

As mentioned above, the pathology is associated with MHC or HLA in humans. They are groups of genes located on the short arm of chromosome 6 that encode for three types of molecules: class I, II, and III. Class I

have been related to ankylosing spondylitis. HLA-B27 is the main gene involved and has 167 known subtypes.[3, 8]

Although the molecule's role has been investigated for almost half a century, its participation is not fully elucidated. It is still known to participate in the endogenous antigens' presentation to cytotoxic T lymphocytes (CD8+) and initiate the immune response. The problem is that its high polymorphism can affect the specificity of binding antigen peptides and the pathogenicity and susceptibility of ankylosing spondylitis.[4, 10] There are several hypotheses about how HLA affects the immune response (**Table 1**).

Studies indicate that patients with positive HLA-B27 phenotype have severe clinical conditions. Similarly, it is linked to earlier disease onset and diagnosis and longer duration. Additionally, there is a high probability of finding the gene in members of the same family with ankylosing spondylitis.[10]

Table 1: Hypothesis about the influence of HLA-B27 on ankylosing spondylitis.[3]

Hypothesis	Mechanism
Arthriotogenic peptide	Self-antigen binds to HLA-B27 subtypes, triggering the immune response. Nevertheless, none have been found capable of binding to all the subtypes involved.
Misfolding of HBLA-B27	HLA-B27 misfolding in the endoplasmic reticulum, which activates endoplasmic reticulum-associated degradation (ERAD). Still, it can occur inefficiently, and defective autophagy in polymorphonuclear cells could happen. The stress occasioned by the misfolded proteins causes an increase in genes whose transcripts increase the capacity to fold proteins correctly or make them enter apoptosis with the release of proinflammatory substances, including interleukin-1 (IL-1), IL-23, and interferon-beta (INF-β).
HLA-B27 free heavy chains	HLA-B27 has a high tendency to misfold and form homodimers in heavy chains that can bind to: -T lymphocytes, increasing their half-life. -Macrophages, causing their differentiation to dendritic cells. -Regulatory T lymphocytes (Treg), augmenting their response. Besides, it stimulates proinflammatory activity in Natural Killer (NK) cells, T cells, and T helper 17 (Th-17) lymphocytes, enhancing the synthesis of IL-17.

The second important gene is ERAP1. It encodes for endoplasmic reticulum aminopeptidase 1, responsible for making cuts in the antigen before the HLA presentation. It has distinct polymorphisms. Some wild-type variants result in an aberrant presentation of antigens, altering the innate response and increasing the disease incidence. In contrast, some polymorphisms show a protective effect. Moreover, this gene is highly related to HLA-B27.[11, 12]

PATHOPHYSIOLOGY

Its pathophysiology is not well described, and the primary trigger for inflammation is unknown. Even so, it embraces several molecules and pathways employed as targets for their treatment. The most related are IL-17 and 23.[12] IL-17 stimulates the synthesis of cytokines such as tumor necrosis factor (TNF), and IL-23 induces the production of interferon-gamma (INF-γ) and memory cells.[13]

In general, this pathway is essential for developing autoimmune diseases driven by T lymphocytes, including ankylosing spondylitis. Furthermore, it is related to the Th17 lymphocytes subpopulation.[14]

IL-23 also promotes pathogenesis, stimulating helper T cells (CD4+) differentiation of the Th17 subpopulation. This situation produces an IL-17 increase, mainly the more pro-inflammatory subtypes (IL-17A/A and IL-17A/F). Similarly, it stimulates endothelial cells, epithelial cells, and fibroblasts. Such cells secrete cytokines (IL-6, IL-8, and TNF) considered relevant inflammatory mediators.[14]

In the case of TNFα, it is a highly pro-inflammatory cytokine and essential in inflammation development. It stimulates other cytokines' production and increases the immune cell's incorporation in the affected joints, augmenting the damage.[15]

DIAGNOSIS

The disease is measured by the patient's functional deterioration and the severity on X-rays. It is generally measured by the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS). In addition, it should be observed in the images if there are sacroiliitis presence and the degree of inflammation and pain.[16]

Exclusive use of radiographs can delay the diagnosis. Its interpretation is not straightforward and takes a long time for the sacroiliitis to be observed. Therefore, magnetic resonance seems to have a more significant advantage for an early diagnosis, allowing to appreciate inflammatory processes in the spine and sacroiliac joints and edema in the spinal cord. Also, it allows evaluating the response to treatments with different TNFα inhibitors, such as etanercept, adalimumab, and infliximab. As a complement, it allows the spinal inflammation assessment according to the Braun scoring system called ASspiMRIa (ankylosing spondylitis spinal magnetic resonance imaging activity).[17, 18]

The most known parameters are the New York criteria and the Bath Ankylosing Spondylitis Radiology Index (BASRI). They are based on the scoring of the various clinical manifestations by radiological image analysis. Plus, the questionnaire application to patients determines the general condition of each one. The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) estimates disease activity for axial pain, fatigue, morning stiffness, and peripheral joint disease. The Bath Ankylosing Spondylitis Disease Functional Index (BASFI) determines the physical capacity and functional ability in daily life. Besides, the Ankylosing Spondylitis Quality of Life questionnaire (ASQoL) assesses life quality.[19]

TRADITIONAL TREATMENT

The first-line treatment involves NSAIDs. These drugs reduce the symptoms of spine-impaired mobility. Furthermore, studies have shown its ability to provide rapid relief from inflammatory back pain. For spondylitis treatment, they have more significant potential as anti-inflammatory drugs than analgesics, working to control spinal stiffness and improve physical function. The drugs include indomethacin, diclofenac, ibuprofen, naproxen, celecoxib, and etoricoxib.[20, 21]

One advantage is the rapid-acting and substantial symptomatic effect, which explains its utilization as a potential tool to diagnose the disease. Sufficient back pain relief in the first 48 hours after its administration or rapid relapse of pain after its discontinuation is taken as a diagnostic criterion for spondyloarthropathy.[20]

Nonetheless, they have several disadvantages. Often, they fail to control symptoms in the long term, and the patient stops responding to this initial treatment. In addition, after withdrawal, rebound inflammatory symptoms (pain and swelling) are seen within a few days, and there is no evidence that they change the pathogenetic mechanism. Thus, joint damage can continue despite symptomatic improvement.[20]

As a result of their chronic use, there are second-generation NSAIDs with less toxicity. The difference with those of the first generation is their selectivity for the cyclo-oxygenase 2 (COX-2) enzyme, expressed in some cells under stimuli like chemical mediators of inflammation. Among the drugs preferentially inhibiting COX-2 are celecoxib and meloxicam.[22]

Even so, its employment is limited by gastrointestinal adverse effects, particularly COX-1 inhibition. This enzyme is responsible for the production of cytoprotective prostaglandins in the gastric mucosa.[20]

Moreover, the side effects of NSAIDs may include gastroenteric toxicity (increased dyspepsia), cardiovascular risk, hypertension (especially in patients where blood pressure control is difficult), and renal effects (electrolytes abnormalities, acute renal failure, nephrotic syndrome, renal papillary necrosis, and edema because of sodium and fluid retention).[21]

In the case of glucocorticoids, prednisone is highly effective in relieving symptoms and can delay joint injury. It can be administered alone or with NSAIDs in the early disease stages of rheumatic diseases.[23]

The second-line treatment comprises modifying antirheumatic drugs. They should be considered when a patient does not respond well, has many adverse effects to NSAIDs, or is in an advanced, severe disease stage. The traditional modifiers are methotrexate, hydroxychloroquine, leflunomide, sulfasalazine, and azathioprine. They are an effective therapeutic alternative by inhibiting the immune system, achieving an anti-inflammatory and immunosuppressive activity, and modifying the disease course.[20, 24]

Methotrexate is very helpful as it suppresses synovitis (joints inflammation). Additionally, it has an immunosuppressive action, acting at the cellular level on cytokines.[23, 24]

For its part, hydroxychloroquine is an antimalarial medication. This product impedes prostaglandins and lysosomal enzymes' release, lymphocytic proliferation, immunoglobulins production and promotes antigen processing modification.[23]

As for leflunomide, it is an inhibitor of an intracellular enzyme necessary to synthesize the pyrimidine by activated lymphocytes. Thanks to this, it delays the radiological progression of the joint injury. Still, it has a long half-life, increasing the side effects.[23, 24]

Regarding sulfasalazine, it belongs to the sulfa drugs group. It has anti-inflammatory and immunomodulatory action. It is used when methotrexate and leflunomide treatments are not available.[24]

Finally, azathioprine is a derivate of 6-mercaptopurine that has liver metabolism to become active. This drug inhibits the synthesis of DNA and RNA. Also, it blocks lymphocyte production *in vitro* and IL-2 production, action related to its antiproliferative activity.[25]

Despite the benefits provided, these drugs have many side effects. With the problems linked to these therapeutic options, it is necessary to consider biological therapies as a third-line treatment. The best-known option considers drugs that block TNF. It should be noted that a failure with the NSAIDs must be reported before providing anti-TNF treatment to patients with active disease.[21] This alternative will be reviewed below.

BIOLOGICAL THERAPY AS AN OPTION AGAINST ANKYLOSING SPONDYLITIS

According to the Regulation for the Registration and Control of Biological Medicines of Costa Rica, biological drugs are defined as those manufactured from microorganisms, animal, or vegetal organs or tissues, animal, human, or animal cells or fluids, cell designs, and products of biotechnological origin.[26] Among them are blood products, vaccines, sera, antibodies, recombinant proteins, cytokines, interferons, gene therapy, cell therapy, and DNA and mRNA molecules.[27, 28] Many are obtained through modifications in the genome of viruses, bacteria, animal or plant cells. For this, recombinant DNA technology is employed. The latter products are known as biotechnology products.[27]

By their nature, these drugs are molecules that are complex, large, and with little physicochemical stability. They can change their composition, affecting the safety and efficacy in treating several conditions.[28]
Monoclonal antibodies are a group of biotechnological drugs. They have been studied for autoimmune diseases, and specifically rheumatic diseases such as ankylosing spondylitis.

GENERAL ASPECTS OF ANTIBODIES

Antibodies are glycoproteins whose primary biological function is to bind to an antigen that has entered the body, marking it to facilitate recognizing by an immune cell capable of its destruction. The advance in their knowledge is described in **Table 2**. Antigens have a delimited section recognized by antibodies called epitopes. The same antigen can have several epitopes and cause a wide range of antibodies by the body's B lymphocytes (polyclonal antibodies).[29]

Table 2: Historical advances on the development of monoclonal antibodies.[30, 31, 32, 33]

Year	Advance
1700's	Edward Jenner developed the first vaccine (smallpox) by inoculating the fluid in the pustules of cows (cowpox) in humans, generating immunity to the human virus.
1890	Kitasato and von Behring noted that serum from guinea pigs immunized with diphtheria contains antitoxins, eliminating bacterial toxins. The antitoxins were later named antibodies by Behring.
1923	Avery and Heidelberger discovered that antibodies are proteins.
1934	Marrack described the antigen-antibody complex formation and shows that most antibodies are divalent molecules.
1945	Harris, Ehrich, Grimm, and Mertens determined that lymphocytes play a fundamental role in antibody formation.
1972	Porter and Edelman received the Nobel Prize for elucidating the chemical structure of antibodies.
1975	Jerne, Köhler, and Milstein created the hybridoma technology, fusing a murine non-secretory myeloma cell with a B-lymphocyte clone of an immunized mouse producing the antibody of interest. They developed the first monoclonal antibody.

Due to multiple investigations, it was discovered that *in vitro* antibody-producing cell clones could be formed by the fusion of plasma cells (from a mouse spleen) immortalized in B cell myelomas. Identical offspring can produce a single type of antibody (monoclonal antibodies). Through Milstein and Köhler's discovery, these molecules could be directed against specific targets.[29]

CHARACTERISTICS OF MONOCLONAL ANTIBODIES

The first technique to obtain monoclonal antibodies required that a mouse was immunized with a given antigen. Then, the lymphocytes that produced the corresponding antibodies were taken from the spleen and fused with myeloma cells, creating hybridomas. The hybridoma takes from the lymphocyte the ability to produce antibodies and from the myeloma cell the capacity to reproduce indefinitely in a culture media.[34]

With this strategy, the first monoclonal antibody authorized in humans, specifically for kidney transplant rejection, was muromonab-CD3. It is a murine molecule directed against the CD3 (cluster of differentiation 3) antigen on mature peripheral human T cells. The molecule blocks the function of these cells. Its mechanism of action involves antigenic modulation of the CD3/TCR (T cell receptor) complex, with subsequent opsonization and elimination of circulating T cells. However, murine antibodies produced human anti-murine antibodies (HAMA) in many patients, developing severe side effects such as nephrotoxicity and/or anaphylactic reactions.[35, 36]

To overcome this drawback, chimeric antibodies were generated through recombinant DNA technology. The genes that encode the variable region of mouse immunoglobulins G (IgGs) were joined with the constant human region's genes.[33] In this way, the antibody combines the antigen-binding parts (antigen-binding fragment or Fab) present in the variable region of the murine antibody with the effector portions (crystallizable fragment or Fc) found in the constant region of the heavy chains of a human antibody (70 % human molecule).[37] The first chimeric antibody was obtained in 1985.[33]

Through this, the development of humanized monoclonal antibodies was encouraged. They combine the antigenic specificity of mouse immunoglobulin with the other properties of a human immunoglobulin. The result was approximately 95 % human molecules.[37]

Development and improvement in this field went as far as fully human monoclonal antibodies. Humanized and fully human antibodies have a meager immunogenic potential and show functional properties like endogenous human IgG.[29] All the above information, including the way to name them, is summarized in **Table 3**.

Currently, they constitute a fundamental element in biomedical research. The drugs' functions comprise inhibiting therapeutic targets for treating pathologies such as cancer, arthritis, psoriasis, asthma, and transplanted organ rejection.[36]

Table 3: Composition of the distinct types of monoclonal antibodies.[36, 37]

Monoclonal antibodies	Composition	Suffix
Murine	Modified mouse antibodies.	-omab
Quimeric	Murine sequences in the variable regions of human antibodies.	-ximab
Humanized	Approximately 90 to 95 % of the human sequence.	-zumab
Fully human	100 % human sequence.	-umab

OBTAINING MONOCLONAL ANTIBODIES

The development of monoclonal antibodies occurs by diverse methods. They are briefly mentioned and explained.

Hybridoma generation

This process begins with the fusion of a B lymphocyte from an animal immunized with the antigen of interest (lymph nodes or spleen) and a non-secretory myeloma cell (using polyethylene glycol or PEG) without the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT). The cells are cultivated with hypoxanthine, aminopterin, and thymidine (HAT media). It does not allow unhybridized myeloma cells and unfused B lymphocytes to survive, leaving only the hybridomas. Subsequently, the supernatant specificity of the culture plates is analyzed, selecting only the desired ones. Then, positive hybridomas are cloned by limiting dilution. If necessary, they are frozen in liquid nitrogen plus dimethyl sulfoxide (DMSO) and fetal bovine serum (FBS) and utilized later. It is considered one of the most suitable production methods against a known antigenic determinant.[38, 39]

Transgenic animals

This procedure reduces adverse effects and increases therapeutic effectiveness. They are successor hybrids of a first progenitor mouse strain in which the genes necessary for immunoglobulins production have been inactivated. The locus and loci of heavy and light chains are introduced in the second progenitor in its antibody-producing cells. With this, a strain that expresses only human immunoglobulins is achieved.[38, 39, 40]

Phage therapy and ribosomal therapy

Phage therapy involves presenting proteins on the surface of filamentous phages to achieve monoclonal antibodies without mice's use. Through recombinant DNA techniques, genes from any organism are introduced into its genome. Thereby, the viruses express various therapeutic proteins on their surfaces. An attempt to obtain a molecule that retains the maximum affinity for a specific therapeutic target is made. Multiple genetic combinations of the molecule are done, and each one fuses with the gene that encodes the phage capsid proteins and is expressed on its surface (phage clones). Subsequently, those that express the molecule with the best binding properties to the therapeutic target are chosen.[41]

For its part, ribosomal therapy implicates the generation of monoclonal antibody fragments *in vitro*. The technique creates antibody genes through the synthesis of their fragments with ribosomes. The *in vitro* environment can be manipulated and optimized for gene expression, folding, and stability.[38]

At present, instead of bacteriophages, it is preferred to utilize phagemids. They are artificial plasmid vectors made up of gene III of the phage, genomic sequences essential for *Escherichia coli* infection, and the antibody of interest's DNA sequence. The polymerase chain reaction (PCR) makes it possible to obtain all the constituent phages of human monoclonal antibodies of the antibody fragments or phage display libraries.[37]

Additionally, the available recombinant technology allows the manufacture of antibody-derived fragments such as bivalents, trimers, or tetramers. Problems related to the complete antibody molecule are solved, facilitating the binding to individual therapeutic targets.[39]

INDUSTRIAL PRODUCTION

Once the monoclonal antibodies have been obtained, they proceed to the two phases of industrial production. The elaboration or upstream phase begins with the cells' cultivation to produce the therapeutic protein on a small scale, using suitable media for their growth. The cell line is preserved in vials, creating a cell bank. After each step, the containers have more volume of culture media during the scaling process. Cells are constantly dividing in a favorable environment, and strict controls are required to guarantee the product's quality and reproducibility, including temperature, pH, oxygen level, and nutrient concentration. Likewise, tests for bacteria and yeast contamination are performed continuously, as their presence affects the batch. Cells with a greater capacity for stable growth in various cultures are transferred to higher volume equipment, and the production capacity and quality of the protein are assessed.[41]

The process is then developed in the downstream or transformation phase. The monoclonal antibody is isolated and conducted through continuous filtration and purification processes (critical influence on process economics, due to throughput). The product is then formulated to current specifications and prepared for clinical use. The production steps are specific for each drug.[41, 42]

The most frequent technology for its purification from animal cell cultures is column chromatography techniques, specifically affinity. The antibody generation's antigen is covalently attached to the column matrix, and the solution to be purified is passed through it. Next, the specific antibodies for the retained antigen are bound, and the rest of these molecules and contaminants present are removed. After that, the antigen-antibody bonds are broken, adding a hypertonic solution or one at acid pH. When broken down, specific proteins are obtained from the bottom of the column.[37]

As a complement, some monoclonal antibodies' production is explained, and later, their employment in ankylosing spondylitis is described. For adalimumab, the commercialized biological product's manufacturing process has been modified to increase robustness, introduce new technology, utilize alternative raw materials suppliers, and change the production scale. Its progressive demand, generated from the expansion of the indications and the patients' requirements, has necessitated augmenting the manufacturing capacity through production scale increases and new manufacturing sites. These changes on the product's properties had to be evaluated and the quality record maintained. The characteristic fingerprint of adalimumab, comprising a heterogeneous population of molecular species, has been well recognized and has not changed significantly throughout the clinical and commercial product life.[43]

Besides that, according to the manufacturer's recommendation, the culture media is typically prepared for infliximab using a well-established biphasic culture protocol for recombinant Chinese hamster ovary (CHO) cells. In the first stage, they are culture at 37 °C to maximize the growth rate. In the second step, once the maximum cell density has been reached, the temperature is reduced to 33 or 31 °C, minimizing additional growth while reducing the product degradation and favoring the general productivity.[44, 45]

Regarding golimumab, its production occurs through recombinant DNA technology using the Sp2/o cell line. It comes from mammalian cell cultures capable of making modifications required for this protein.[46, 47]

Finally, secukinumab production requires NSO (murine myeloma) and CHO cells. CHO cells are the most widely considered because of their great versatility and production, but they produce tolerance problems in patients due to glycoforms. These structures can generate anti-antibodies against the drug.[46, 48]

MONOCLONAL ANTIBODIES COMMERCIALIZED FOR THE TREATMENT OF ANKYLOSING SPONDYLITIS

Currently, six drugs are marketed to treat ankylosing spondylitis. They have two therapeutic targets: TNFα and IL-17. TNFα inhibitors include infliximab, adalimumab, golimumab, and certolizumab pegol. Furthermore, those with an anti-IL-17 sanitary registration are secukinumab and ixekizumab.

Anti-TNFα

Anti-TNF drugs are directed at a protein responsible for generating inflammation. Its suppression reduces joints inflammation, helping to prevent damage. Nevertheless, it is more difficult to fight infections.[49]

For ankylosing spondylitis, TNFα plays a crucial role in its pathogenesis. By neutralizing this cytokine, an effective anti-inflammatory therapy that preserves the joint structure has been observed, slowing its interaction with the tumor necrosis factor receptor 1 (TNFR1) and therefore, preventing the biochemical cascade that triggers the activation of nuclear factor kappa B (NFκB).[50, 51]

NFκB is a protein complex in animal cells involved in the cellular response generated by stimuli such as stress, cytokines, radiation, and antigens. Another effect of blocking TNFα is the change in adhesion molecules' production levels responsible for leukocyte migration, such as ELAM-1, VCAM-1, and ICAM-1. They are molecules recognized for playing relevant roles in inflammation and immune responses. There is also a decrease in the matrix metalloproteinases levels, responsible for tissue remodeling.[51]

Plus, anti-TNFα drugs correlate with Dickkopf-1 (DKK-1) serum levels, a protein related to new bone formation in ankylosing spondylitis. DKK-1 accelerates the bone formation resorption process through the Wnt pathway (related to various diseases and normal bone metabolism). It has been observed that in mice that overexpress a truncated form of TNFα, DKK-1 blockade results in decreased erosions and counts of osteoclasts, multinucleated cells whose primary function is the osteoformation and maintenance of hematopoietic stem cells. Another action is the regulation of bone resorption by modulating the receptor that activates the NFκB ligand, as mentioned previously.[52, 53]

Commercialized anti-TNFα drugs treat other illnesses like psoriatic arthritis, rheumatoid arthritis, and ulcerative colitis.[51]

Infliximab

Infliximab is a chimeric monoclonal antibody composed of a murine variable region and a human IgG1 constant region. The drug specifically inhibited the TNFα activity and interacted with this soluble cytokine's monomeric and trimeric forms.[54]

Its employment has shown significant improvements in the activity of ankylosing spondylitis.[55] Treatment efficacy is measured according to the ASAS20 and ASAS40 improvement criteria, defined by the Assessment of SpondyloArthritis International Society (ASAS). These criteria evaluate four aspects: the patient's general condition, pain, function, and inflammation. Three of the four domains assessed should improve by 20 % according to ASAS20 and 40 % in ASAS40.[56]

The clinical studies' most relevant results comparing the efficacy of infliximab versus placebo revealed an ASAS20 at week 12 of a 61.9 % response to treatment versus a 20.5 % response to placebo, and an ASAS40 of 49.3 % versus 12.8 %, respectively. Meanwhile, another clinical study demonstrated an ASAS20 response to infliximab of 68.8 % versus a response of 27.2 % for placebo at week 12.[57, 58, 59]

Adalimumab

It is a fully human IgG1κ monoclonal antibody. It is aimed at neutralizing TNFα by blocking the receptor subunits p55 and p57 on the cell surface. In this way, it interrupted the sequential activation cascade of inflammatory pathways mediated by this cytokine.[54, 60]

The most relevant phase III clinical studies performed a comparison against placebo. The results showed that adalimumab reduced the signs and symptoms of active ankylosing spondylitis.[58] Besides, they demonstrated the treatment efficacy concerning the results obtained at week 12. In a clinical study during this period, the ASAS20 reported a 58.2 % response for adalimumab and a 20.6 % in placebo, while the ASAS40 response was 39.9 and 13.1 %, respectively. Meanwhile, a second investigation revealed an ASAS20 response rate of 47.4 % for adalimumab and 27.3 % for placebo at week 12, and an ASAS40 of 44.7 % versus 9.1 %, respectively. Finally, another clinical study exhibited a similar trend from the previous results. Adalimumab achieved an ASAS20 of 67.2 % versus 30.4 % for placebo at week 12, and an ASAS40 response of 44.5 % versus 9.6 %, respectively, in the same period.[58, 61, 62, 63]

Golimumab

It consists of a fully human IgG1 monoclonal antibody against TNFα. It is approved as an intracutaneous injection in adult patients.[54, 64] This treatment was approved for ankylosing spondylitis in 2009, showing safe and effectiveness throughout a phase III-controlled trial.[65]

Among the most relevant results, the ASAS20 and ASAS40 criteria were compared at 12 weeks. In one of them, an ASAS20 response of 49.1 % was obtained for the drug and 24.8 % for the placebo, and an ASAS40 of 26.9 % in response to golimumab compared to 9.6 % for the placebo. Additionally, according to another investigation, the drug reached an ASAS20 of 59.4 % compared to 21.8 % for the placebo in week 12, and, in this same period, ASAS40 of 44.9 and 15.5 % were obtained, respectively.[58, 66, 67]

Certolizumab pegol

It is a PEGylated Fab' fragment of an anti-TNFα human monoclonal antibody. It lacks the Fc region. The antibody's hinge region is covalently bound to two cross-linked 20 kDa chains of PEG, for which it is called certolizumab pegol. PEGylation is a strategy that provides a series of advantages, such as protection of antigenic epitopes, reduction of the product degradation, stability increase, and improvement of the pharmacokinetic and pharmacodynamic characteristics.[54, 68, 69, 70, 71]

According to a phase III clinical study, in which the efficacy of two-dose treatment (200 mg and 400 mg) was compared with placebo, at 12 weeks, an ASAS20 response of 56.9 % was achieved with certolizumab 200 mg, 64.3 % with 400 mg of the monoclonal antibody and 36.8 % with placebo. Likewise, at 12 weeks, the ASAS40 criterion reported was 40.0, 50.0, and 19.3 %, respectively.[58, 72]

Anti-IL-17

An essential pathway for the disease pathophysiology is IL27/IL23. New therapies have been directed at these inflammatory cytokines and have shown benefit in general inflammatory conditions.[73] Patients generally have both molecules increased, as indicated above, because they are necessary to develop clinical manifestations. Therefore, symptoms are reduced by inhibiting IL-17.[74].

Secukinumab

It is an inhibitor of IL-17A with significant efficacy in reducing disease symptoms. This medication was the first non-anti-TNFα biologic approved for ankylosing spondylitis. It is a fully human IgG1k monoclonal antibody. It is made up of two light and two heavy chains. The latter is fully glycosylated at Asn307.[75,76,77,78]

According to data from phase III clinical trials, the efficacy evaluation at two distinct doses against placebo reported an ASAS20 response at 12 weeks of 59.7 % for 75 mg, 60.8 % for 150 mg, and 28.7 % for placebo. The ASAS40 criterion had a respective scope of 33.1, 41.6, and 13.1 % in this same period. In another investigation, an ASAS20 response of 41.1 % for 75 mg, 61.1 % for 150 mg, and 28.4 % for placebo was reported at 12 weeks. Besides, the responses corresponded to 26.0, 36.1, and 10.8 %, respectively, for the ASAS40 criterion.[58, 79]

Ixekizumab

It is a humanized IgG4 monoclonal antibody that binds to IL-17A and inhibits interaction with the IL-17 receptor.[80,81] It is also approved by the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for psoriasis and psoriatic arthritis. Some studies demonstrate efficacy and safety in patients with active ankylosing spondylitis who have not received any biologic disease-modifying antirheumatic treatment and those who have had an inadequate response or intolerance to TNFα inhibitors.[81,82] Phase III studies compared to placebo showed that over 50 % of people achieved an ASAS40 response at week 16.[81, 83]

DRUGS CURRENTLY UNDER INVESTIGATION

IL-17 inhibitors

Brodalumab

Fully human IgG2 monoclonal antibody that blocks the IL-17A receptor and inhibits the action of IL-17A homodimers, IL17A/F heterodimers, and IL-17E. It also appears to inhibit IL-17C.[84, 85] IL-17 cytokines are a family consisting of six proteins: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F. IL-17A, commonly called IL-17, is the best characterized. IL-17F is 55 % homologous to IL-17A, and they are often co-expressed. Interleukins 17B, 17C and 17D share between 23 and 29 % homology. In contrast, IL-17E has only a 16 % homologous sequence.[86,87]

IL-17A is a potent inducer of IL-6 and IL-8 by fibroblasts. Pro-inflammatory effects have been observed on many cellular targets, including epithelial and endothelial cells, fibroblasts, osteoblasts, and macrophages. The other isoforms are much less described. IL-17D can be expressed by resting CD4+ T cells and at low B cell levels. Little is known about IL-17B and IL-17C, but their functions differ from those of IL-17A. The latter induces the secretion of IL-6 in human fibroblasts, while IL-17B and IL-17C generate the release of TNFα and IL-1b in the THP-1 cell line (IL-17A has a lesser effect on these cells). IL-17E has been involved in diseases, promoting Th2-mediated immunity.[88]

This antibody has been influential in treating psoriatic arthritis and has been approved for psoriasis in the United States, Japan, and Europe.[89] Many clinical trials to evaluate its efficacy have been canceled for ankylosing spondylitis due to safety problems related to suicidal ideas.[85] Nonetheless, a phase III clinical trial data demonstrated an ASAS40 response of 43.8 % at 16 weeks, significantly higher than placebo (24.1 %).[90,91,92]

Bimekizumab

Bimekizumab is a humanized IgG1 monoclonal antibody that selectively neutralizes IL-17A and IL-17F without affecting IL-17E (it has anti-inflammatory effects).[93, 94] Phase II clinical studies reported that its utilization in patients with active ankylosing spondylitis generated rapid and significant ASAS40 responses (46.7 %) at week 12 compared to placebo (13.3 %). This data showed it as a promising therapy option for this pathology.[89, 93]

IL-23/12 inhibitors

IL-23 plays a central role in several inflammatory diseases, including ankylosing spondylitis. It is a heterodimeric cytokine that shares the p40 subunit with IL-12. Both are produced in large quantities by antigen-presenting cells. IL-12 is an essential factor in the differentiation of naïve T cells into IFN-γ-producing Th1 lymphocytes, a mediator of immune reactions. Besides, IL-23 is involved in the differentiation and expansion of Th17 cells, the primary producer of pro-inflammatory cytokines (IL-17A).[95, 96, 97]

According to one study, polymorphisms presented in the IL23R gene, which encodes for the IL-23 receptor, are associated with susceptibility to ankylosing spondylitis. HLA-B27 misfolding is related to increased IL-23 production, leading to augmented activation of Th17 cells. In addition, higher levels of IL-23 have been found in peripheral blood and synovial fluid in patients with ankylosing spondylitis. The p40 subunit, present in IL-12 and IL-23, is necessary to develop T-cell-mediated autoimmune diseases.[95, 96, 97]

Consequently, both IL-12 and IL-23 are part of new drug targets for this illness treatment.

Ustekinumab

It is a fully human IgG1κ monoclonal antibody. It binds with high specificity and affinity to the p40 protein subunit of IL-12 and 23, preventing its binding to the IL-12Rβ1 receptor, expressed on immune cells' surface. It has been effective in conditions such as psoriasis, psoriatic arthritis, and Crohn's disease. As a result of the similarity in these diseases' pathogenesis, studies have been conducted to evaluate patients' safety and efficacy

with active ankylosing spondylitis. Up to 65 % of individuals achieved an ASAS40 response in week 24, and up to 75 % reached the ASAS20 criterion in the same week.[98, 99]

Risankizumab

It is a humanized IgG1 monoclonal antibody. It selectively inhibits IL-23 by targeting the p19 subunit. It has revealed efficacy and safety in ankylosing spondylitis, according to a phase II clinical study. However, the evidence is few, so its employment is not approved.[100]

OTHER BIOLOGICAL AGENTS

JAK inhibitors

Janus kinases (JAKs) make up a family of 4 members: JAK1, JAK2, JAK3, and TIK2. They are tyrosine kinase proteins that autophosphorylate or transphosphorylate tyrosine residues, including signal transducers and activators of transcription (STATs), a group of transcription factors.[101] The JAK/STAT pathway mediates the effect of molecules such as interleukins, interferons, growth factors, and hormones. By binding to type I and II receptors associated with JAKs, they phosphorylate STAT molecules, regulating genes' expression.[98, 101] The route has attracted interest as a potential treatment for inflammatory diseases. Therefore, it is considered a strategy for its consideration in patients with ankylosing spondylitis.[102]

An example is tofacitinib. It is a first-generation drug, an inhibitor of JAKs, with a preference for JAK1 and JAK3 and functional selectivity over JAK2.[101, 103] The inhibition affects the signaling pathway of some interleukins such as 17, 21, and 23. Also, it reduces serum levels of TNF.[103, 104]

It is approved for rheumatoid arthritis in several countries, and its effect and safety in other conditions such as psoriatic arthritis and ankylosing spondylitis are being studied. According to phase II clinical studies, 5 mg every 12 hours achieved an 81 % ASAS20 response and 46 % in ASAS40 at week 12, compared with 41 and 20 % response to placebo, respectively.[102, 103]

Filgotinib is another selective JAK1 inhibitor that is being evaluated for ankylosing spondylitis. In one phase II clinical study, its efficacy and safety were tested against a placebo. The results indicated 76 % ASAS20 and 38 % ASAS40 responses for the drug at week 12, verses 40 and 19 % for placebo, respectively.[105]

Likewise, upadacitinib is a selective JAK1 inhibitor. It has been assessed in other inflammatory diseases such as Crohn's disease, psoriatic arthritis, ulcerative colitis, and atopic dermatitis. A phase II/III study obtained a 52 % response with ASAS40 against a 26 % achieved with placebo at week 14. Likewise, 65 % of the ASAS20 criterion was found with upadacitinib versus 40 % with placebo in this same period.[106]

Regulatory T (Treg) cells

Treg cells play a relevant role in immune tolerance and autoimmunity prevention.[107] They are a subpopulation with phenotypic and functional characteristics of suppressor cells that regulate the immunological activity. The FoxP3 transcription factor expression is decisive for Treg lymphocytes' differentiation, being an exclusive marker that defines its function as a suppressor cell.[108, 109]

Recent research shows that ankylosing spondylitis is associated with T lymphocytes through CD4+ and its subsets. FoxP3-positive Treg cells appear to achieve a role in the etiology of ankylosing spondylitis. The mean fluorescence intensity of FOXP3 in circulating Treg lymphocytes decreased significantly in patients with active disease. Because of this, FOXP3 deficiency is associated with severe autoimmune and proliferative disorders.[109, 110, 111]

Phases I and II clinical trials have only been made in transplant or cancer patients to evaluate its modulation. However, the functional defects found in this autoimmune pathology suggest its relevant role and may become a therapeutic target.[107, 111]

CONCLUSIONS

Ankylosing spondylitis is a disease whose management is complicated. Despite various drugs within the traditional therapeutic lines for its treatment, many side effects are associated with their use, and the disease progression continues, even do the illness symptoms are controlled.

Thus, monoclonal antibodies constitute an unquestionable therapeutic tool, which, together with the relevant advances of technology and molecular biology, allow their interaction on critical pharmacological targets in the etiopathogenesis of severe and even fatal diseases, including ankylosing spondylitis. Although its pathophysiology is not well described, some immune system elements have been found on which different biological therapies can be considered, mainly monoclonal antibodies against TNFα (infliximab, adalimumab, golimumab, and certolizumab pegol) and IL-17a (secukinumab and ixekizumab). There are also many other monoclonal antibodies and molecules in various stages of research.

Nevertheless, there is no 100 % effective biological drug. The current scenario opens the possibility for future research on the subject to develop more therapeutic options. In the future, it is expected to have these drugs to deal with a chronic disease capable of being disabling and even lead to a permanent condition when it has advanced significantly.

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