Reumatol Clin. 2009;**5(1)**:34-39

Reumatología Clínica

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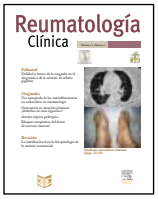

Review Article

Interleukin 6 in the Physiopathology of Rheumatoid Arthritis

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ARTICLE INFO

Article history:
Received September 17, 2008
Accepted October 21, 2008

Keywords:
Interleukin 6
Cytokines
Rheumatoid arthritis
Physiopathology
Therapy
Tocilizumab

ABSTRACT

Interleukin (IL) 6 was identified in 1986 as a factor produced by T lymphocytes, that mediates growth and immunoglobulin synthesis on B lymphocytes. IL-6 is a member of a large cytokine family sharing a gp130 membrane receptor. This receptor mediates specific Jak/STAT3 activation, which induces widespread expression of pro-inflammatory and immunoregulatory genes. IL-6 mediates potent systemic responses, in organs distant from its local inflammatory sources, in a prominent fashion compared to other cytokines. Most specific effects involve hematopoiesis and hepatic acute phase reactants synthesis. IL-6 became a rheumatoid arthritis (RA) target due to its pro-inflammatory and joint destructive potential, as well as its participation in T and B immunoregulation. The therapeutic success of tocilizumab has confirmed IL-6 as an RA target.

Although additional studies on the participation of IL-6 in RA physiopathology are needed, a number of indirect data point to a relevant position in this setting.

La interleucina 6 en la fisiopatología de la artrirtis reumatoide

Palabras clave:
Interleucina 6
Citocinas
Artritis reumatoide
Fisiopatología
Terapia
Tocilizumab

RESUMEN

La interleucina (IL) 6 fue identificada en 1986 como un factor producido por los linfocitos T, con efectos estimuladores del crecimiento y síntesis de inmunoglobulinas en los linfocitos B. Pertenece a una amplia familia de citocinas que comparten el receptor de membrana gp130, mediador de una señal específica de activación del sistema Jak/STAT3 con amplios efectos en la expresión de genes proinflamatorios e inmunorreguladores.

De forma más prominente que otras citocinas, la IL-6 media potentes acciones sistémicas en órganos distantes de su origen local inflamatorio. Las más específicas afectan a la hematopoyesis y la síntesis hepática de reactantes de fase aguda. Su potencial actividad proinflamatoria y de destrucción articular, junto con su implicación en la inmunorregulación T y B, la convirtió en una diana terapéutica atractiva, confirmada por el éxito de su antagonista tocilizumab en la artritis reumatoide (AR).

Aunque son necesarios estudios más amplios sobre la participación de la IL-6 en la fisiopatología de la AR, numerosos datos indirectos permiten situarla en una posición muy relevante.

Introduction

In the 1970's, parallel to the general interest of the immunologists in studying antigen recognition and response, some research groups were interested in other, "non-specific" regulating factors, soluble molecules that were generally identified as cytokines. The study of soluble factors which, produced by stimulated T cells cooperate with B cells stimulating antibody synthesis, was critical in the latter identification of interleukin (IL) 6. This group of factors then generally known as TRF (T cell replacing factors). The first to be identified, initially as peptides and then as genes were named BSF (B cell stimulating factors) or BGF (B cell growth factors) and are currently known as IL-4 and IL-5.[1] In 1986, the group led by Dr Tadmitsu Kishimoto identified another of these factors, initially named BSF-2 due to its potent immunoglobulin-synthesis stimulating activity (BSF) and later as plasmocytoma tumoral B cell growth factor (BGF), and is currently known as IL-6.[2]

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Some initial observations of that group contributed to form the profile of a wide spectrum of biologic effects produced to this cytokine. An interesting observation was the detection of IL-6 in the cardiac myxoma tumor cell culture.[3] This tumor is accompanied by clinical manifestations such as fever, anemia, increase in acute phase reactants (APR), and autoantibodies, which disappear after its excision.[4] The identity of IL-6 as a "hormone," acting at a distance, was also confirmed, being known up until that moment as HSF (hepatocyte stimulating factor), capable of inducing the synthesis of proteins as C-reactive protein (CRP) and SAA, implicated in the systemic response to inflammation and denominated RFA.[5] Dr Kishimoto himself described, at the end of the 1980's, the excess of IL-6 in synovial rheumatoid tissue[6] and in another process with an important "systemic" component, Castleman's disease,[7] and then promoted the first successful trials of IL-6 antagonist as therapy in both diseases.

Biology of IL-6

Although IL-6 was identified as a factor produced by T lymphocytes, these are not the only cells to produce it, but also a wide variety of cell types that include macrophages, endothelial cells, glial cells, keratinocytes, or fibroblasts. These seem to be the most abundant cell source of IL-6 in the bone marrow stromal tissue and in the inflamed synovial membrane.[8] IL-6 is a small polypeptide, with homology with a larger family of sytokines: IL-11, neutrophillic cylliar factor (CNTF), cardiotrophin-1 (CT-1), cardiotrophin-like cytokine (CLC), leukemia inhibiting factor (LIF), neuropoietin (NPN), and oncostatin M (OSM), recently widened by the inclusion of IL-27 and IL-31. All of them share the use of a very common cell receptor, present in almost all cells: gp130, capable of transmitting intracellular signals through phosphorylation of its intracellular domain-associated proteins. However, IL-6 is not capable of binding to gp130 in the absence of a second specific receptor known as IL-6R, whose expression is more restricted to hepatocytes, neutrophils, monocytes/macrophages, and some lymphocytes.

The IL-6R receptor presents an interesting characteristic, the fact that it can be found in a soluble form in inflammatory environments, from 2 possible sources: one, the product of alternative splicing of its messenger RNA which generates a variant which lacks the membrane anchoring domain or, a second form, formed by limited protein lysis from the membrane bound form.[9] This proteolysis depends on the ADAM17 proteinase, also known as TACE (due to its membrane bound tumor necrosis factor [TNF]-like effect), which releases the previously neutrophil or macrophage membrane-bound form. This soluble form of IL-6R is capable of binding IL-6 to its functional gp130 receptor, inducing cell signaling in those cells which lack membrane IL-6R, and this mechanism is denominated transignaling (Figure 1).

The type of signal transduction by gp130 is a phosphorylation of an intracellular protein named Jak (Janus kinase) which in turns phosphorylates STAT-3 (a signal transducer and transcription activator), a transcription factor capable of traversing to the nucleus and activating the expression of its target genes.[10] One of them is the SOCS inhibitor gene (cytokine signaling suppressor) which acts as a negative feedback mechanism, interfering with the phosphorylation of STAR3, therefore closing down this cascade (Figure 1). There are many other transcription factors or effector proteins generally related with the immune response and local or systemic inflammation.

Apart from this STAT3 specific signal, activation of gp130 laterally connects with 2 important intracellular cascades, activating them. MAPK (mitogen activated kinases) and that mediated by the lipids IP3K/Akt (inositol phosphatidyl kinase), both of enormous relevance in the induction of proinflammatory and cell survival factors.[10]

IL-6 is generally produced in response to non-specific stimuli, such as bacterial factors (LPS) and other cytokines (IL-1, TNF, PDGF, or even IL-6 itself). IL-6 induces the expression of a transcription factor named NF-IL6, which in turn is a potent inducer of IL-6 expression (Figure 1). This transcription factor also acts in the induction of RFA gene transcription in hepatocytes.

Systemic Effects of IL-6

Cytokines are soluble factors with a range of action which is typically limited to the local environment in which they are produced. However, their entry into the circulation can mediate systemic actions to a certain extent. IL-6 is the most important cytokine with distant or "hormonal" effects. The 3 distant targets which elicit greater interest in the systemic response to inflammation mediated by IL-6 are: the nervous system (fever), liver (APR), and bone marrow (hematologic response), although they are not the only ones.

IL-6 is liberated systemically from the site of inflammation and is capable of reaching and acting on hepatocytes, maybe the most important cell type due to its systemic actions. The hepatocyte expresses both receptors IL-6R/gp130 and therefore is generally sensitive to the effects of IL-6. The effects on liver gene expression are mainly mediated by the NF-IL-6 transcription factor. These effects are nor directly shared by other cytokines, such as IL-1 or TNFα, which by themselves would be incapable of inducing APR, although they can do it by secondarily inducing the expression of IL-6.[11] The effects on the gene expression are a variable sign, either repressors of gene expression for physiological proteins such as albumin, or gene expression activators for proteins known to be APR: fibrinogen, SAA, CRP, etc (Figure 1).

Another liver protein of great interest induced by IL-6 is hepcidin, which causes macrophages to sequester iron deposits and is capable of interfering with the intestinal absorption of iron.[12] This protein is the main mediator of anemia associated to chronic inflammatory diseases. TNFα or IL-1β do not share this effect, who by themselves do not induce this response in the hepatocyte. In addition to this indirect mechanism of anemia, IL-6 has direct actions on hematopoiesis. Megakaryocytes are sensitive to IL-6, which is a mediator of thrombocytosis, although this effect seems to be indirect, mediated through the induction of thrombopoietin, and is common to other inflammation related cytokines. In general, IL-6 directly stimulates the hematopoiesis of the 3 cell lines and lymphopiesis, and the net detectable effects in peripheral blood are leukocytosis, neutrophilia, and thrombocytosis.[13]

Lastly, some locally relevant effects in chronic inflammatory joint lesions, such as bone erosions and possible specific autoimmunity, are also systemic. IL-6 is one of the most important physiopathological factors of bone resorption, participating not only in the systemic osteoporosis which is associated to inflammatory disease, but also in a relevant way in the primary forms. IL-6 synthesis in the bone marrow stromal tissue is modulated both by gene variants as by hormone changes (estrogen deficit) associated to osteoporosis, and participates directly in its pathogeny potentiating osteoclast resorption.[14]

Its effects in the growth and function of B cells is associated to systemic hypergammaglobulinemia and contributes in a disease unspecific manner to the synthesis of autoantibodies such as rheumatoid factor (RF) or ANA, as has been observed in cardiac myxoma.[4] Its potential role in specific autoimmunity has been less studied in humans, although data from animal models indicates that certain autoimmune T and B cell responses, associated to diseases such as arthritis, lupus, or autoimmune encephalomyelitis, can depend on IL-6.[15-17]

Other interesting effects of IL-6 are those related with lipid or glucose metabolism. This, along with its direct participation in inflammatory lesions and endothelial disfunction associated to atherosclerosis, has placed IL-6 in the crosshairs of metabolic and

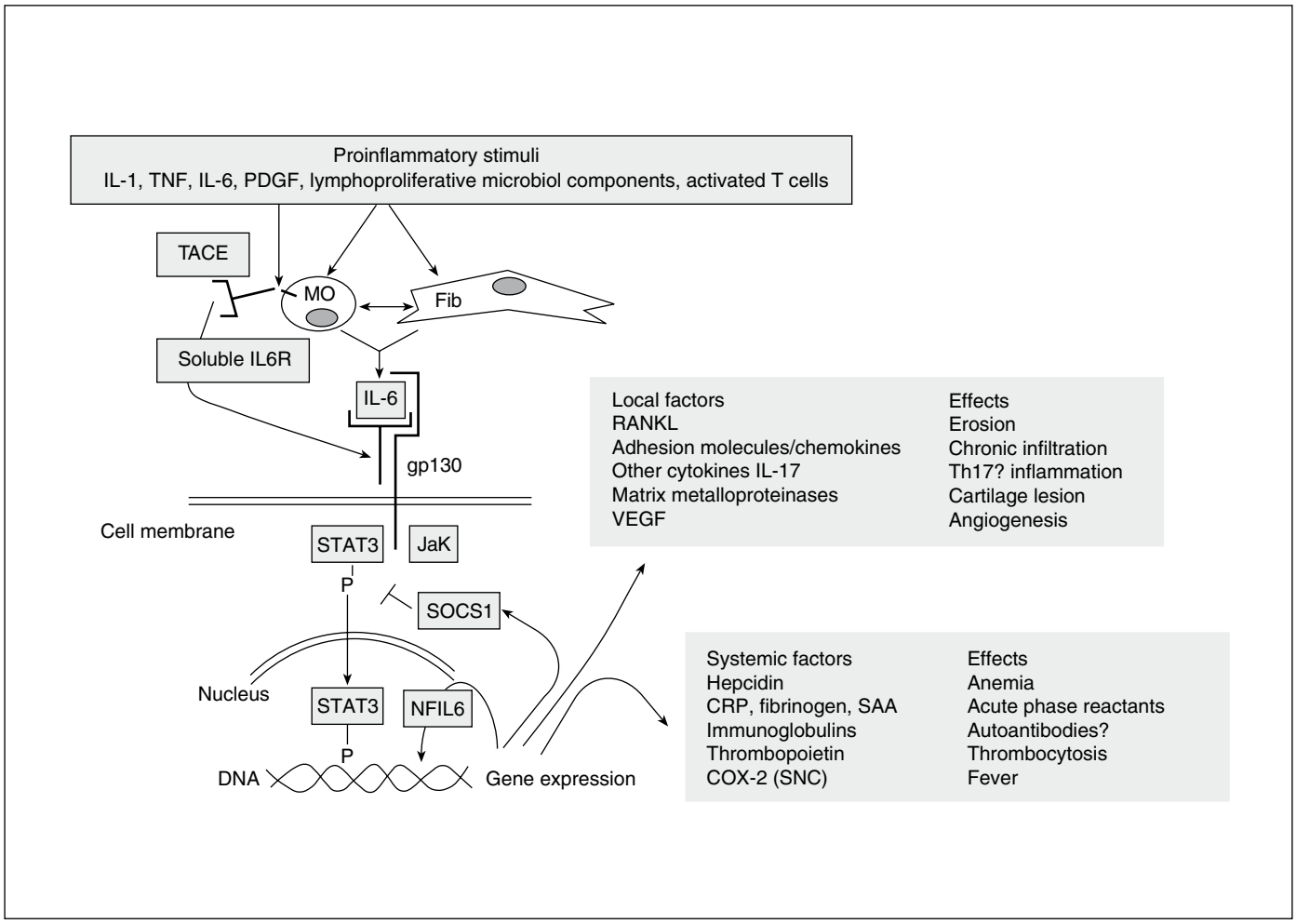

Figure 1. Mechanisms implicated in the biology of interleukin (IL) 6 and its final effects on the physiopathology of rheumatoid arthritis. Cascade of effects and molecules implicated in the production, intracellular signaling and final effects of IL-6 in relation with rheumatoid arthritis. APR indicates acute phase reactants; Fib, fibroblast; MO, macrophage.

vascular disease, and CRP as a good marker of the process. However, data from human physiology and pathology, from animal models based on excess or deficit of IL-6 and from treatment with IL-6 or its antagonists are difficult to integrate, if not contradicting, and the cause-effect relationship are difficult to establish. On one hand, it is important to distinguish the physiological effects of IL-6 derived from its excess in inflammatory diseases. On the other, depending on the animal IL-6 depletion-excess model or therapeutical IL-6 or antagonist protocol analyzed, the effects can be variable or even opposed.

IL-6 from fat tissue seems to be implicated in the vascular morbidity associated to obesity, or the metabolic syndrome, and maybe, next to other cytokines such as TNFα, in that associated to chronic inflammatory diseases such as RA.[18] In contrast, suppression of "physiologic" IL-6 due to knockout in animals, induces obesity and insulin resistance.[19] During exercise, muscle IL-6 is liberated, improving insulin usage and fatty acid oxidation.[20] On the other hand, treatment with IL-6 in patients with cancer reduces the total concentration of cholesterol in all of its lipoprotein fractions, while its antagonists increase them in patients with RA.[21,22] Currently, questions relative to the possible impact of the chronic use of IL-6 antagonists directly in the vascular lesion, indirectly on the metabolic factors and the excess of vascular morbidity and mortality associated to chronic inflammation (RA), represent an important challenge for research.

IL-6 in the Pathogenesis of RA

RA was one of the first inflammatory diseases in which an important increase of the expression of IL-6 was described, both in plasma as in synovial tissue.[7] Its increase in plasma fluctuates rapidly in the same direction as activity, severity, and positive response to therapy, in the same way as its indirect marker, CRP.[23,24] The main effects of the systemic increase of IL-6 in RA are the increase in APR and, therefore, secondary amyloidosis (SAA), chronic disease anemia and possibly, systemic osteoporosis and an increase in vascular risk.

In the synovial tissue, both in the mononuclear cells of the infiltrate as in synovial fibroblasts or synoviocytes, seem to contribute to the excessive synthesis of IL-6.[8] Synoviocyte hyperplasia, the effects of cytokines such as TNF or IL-1 on them and finally a stable phenotypical alteration, contribute to overproduction of IL-6 by these cells, a property that they maintain even when they are cultured ex vivo.[25] Many of the cells implicated in synovitis (chondrocytes, synoviocytes, fibroblasts, endothelial cells) do not have an IL-6R and, however, are sensitive to the effects of IL-6 through a trans-signaling mechanism. There is abundant soluble IL-6R in the joint environment which comes from infiltrating leukocytes, guaranteeing the action of IL-6 on all of these cell elements.[26]

Local or systemic effects of IL-6 on the immune system cells, autoimmune B cells or different populations of T cells are unknown

in the context of RA. However, its function in these cells and its participation in animal models of arthritis are well known in a general manner, making it possible to theoretically predict what IL-6 can do on T or B cells participating in the pathogenesis of RA. As has been pointed out in the history of IL-6, it is capable of inducing the growth and survival of plasma cells and inducing antibody synthesis, 2 effects that can locally or systemically contribute to maintaining the rheumatoid autoimmune response.

Recently, a new form of proinflammatory effector T cells, named Th17 due to their production of IL-17, was described. Differentiation of T cells depends on the cytokine milieu present during the activation by antigen presenting cells.[27] In that way, Th1 depends fundamentally on IL-12; Th2, on IL-4; and Th17 and Treg (regulator) differentiation, on Transforming Growth factor beta (TGFβ) (Figure 2). In the presence of TGFβ, the outcome of the T cell to proinflammatory (Th17) or antiinflammatory (Treg) depends on the fact that the environment presents proinflammatory cytokines or not, fundamentally IL-6. IL-6 not only favors generation of Th17 cells, but blocks differentiation of Tregs and antiinflammatory and immunosuppressive effects.[28,29] Currently, some data points to a Th17 differentiation as a central mechanism which would explain the participation of T cells in RA. IL-17 is an abundant cytokine in the rheumatoid milieu with important, ample spectrum proinflammatory actions on multiple cell types. This, next to the scarce presence in RA of the Th1 and Th2 phenotypes, underlines the potential importance of this effector T cell mechanism. However, the importance of Th17 cells such as IL-6 in its differentiation in RA must be confirmed. Parallel blocking of induced Treg differentiation induced by IL-6 can also be an important factor, because these cells are deficient or dysfunctional in RA and their recovery has been seen during the therapeutic response to anti-TNFα therapy.[30]

The effect of IL-6 antagonists in T and B immunoregulation requires specific immunopathologic tests in RA. We currently only have animal models of arthritis, in which IL-6 contributes to T and B cell responses that cause it and possibly Th17 responses implicated in these models.[15,31] It stands out that, although in these animals TNFα and IL-1 are relevant cytokines which also induce the expression of IL-6, its deficit has a larger protective effect on arthritis than that of TNFα or IL-1.[32]

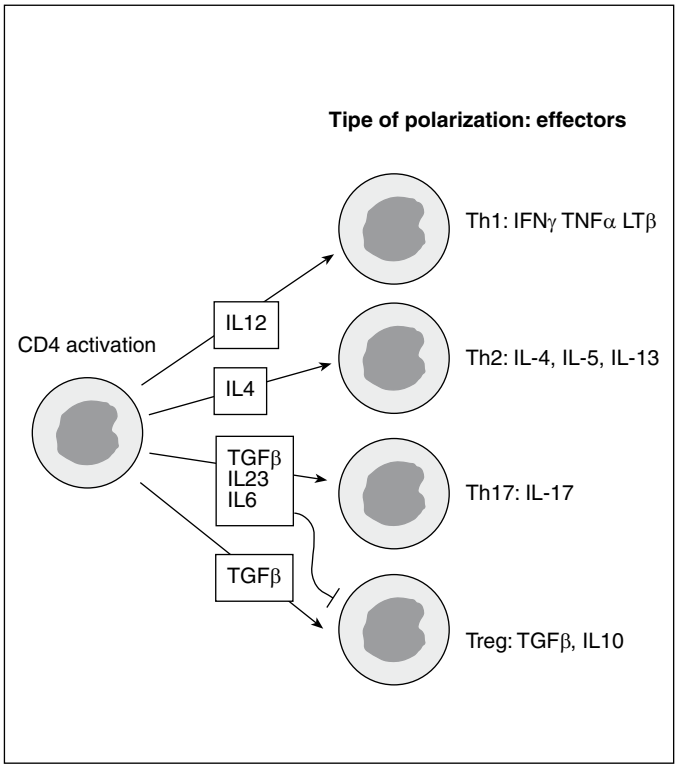

Figure 2. Mediators implicated in the different types of polarization of T cells and their effector cytokines. Participation of interleukin (IL) 6 as a promoter of Th17 cells and proinflammatory or repressor Treg cell differentiation, anti-inflammatory and immunosupressive effects in the presence of TGFβ.

Local Effects of IL-6 in AR

Effector mechanisms of IL-6 in different models of inflammation are well known. Animal models show that IL-6 is not only important in the immunoregulation mechanisms that originate different autoimmune diseases, but is also an important effector of chronic inflammation and tissue destruction, independently of its effects on specific autoimmunity.[15,31-33] An interesting theory with an experimental basis associates the appearance of IL-6 in the site of inflammation with the induction of chemokines such as MCP-1 and other adhesion molecules that change the entry of neutrophils by that of mononuclear cells, something the authors defined as the transition from acute to chronic inflammation.[34] In addition to its ample effects on cell recruitment, acting on endothelial adhesion and inducing the synthesis of different chemokines, IL-6 modifies the response of the different cells that infiltrate the synovium as is described below.

The possible general effects on T and B cells of IL-6 have been mentioned. These effects can also operate locally, modulating the activity of infiltrating lymphocytes in synovial tissue. Another important cell in the local infiltrate are macrophages. Activity of IL-6 on macrophages includes recruitment of precursors, monocytes, and contributions to their differentiation and activation. In this process, an activation of its capacity to ingest bacteria is produced, something with general implications similar to those of TNFα in relation with defense against infection.[35]

The effects of IL-6 on synovial fibroblasts include potential effects on their growth, increasing their survival and modulating effects on the synthesis of other fibroblastic factors such as chemokines, VEGF, or RANKL (Figure 1). In this manner, it can contribute to chronic inflammation (cell recruitment and angiogenesis) and bone erosion. Its direct effects on cartilage are uncertain. Through IL-6 acts on chondrocytes blocking cartilage matrix protein synthesis,[36] its effects are not always concordant in different metalloproteinases or their inhibitors, increasing for example, synthesis of metalloproteinase tissue inhibitor (TIMP).

One of the most studied aspects of bone biology is the osteoclastogenic effect of IL-6, fundamentally through the induction of RANKL in stromal cells and osteoblasts.[14] In addition, IL-6 can directly modulate the differentiation and survival of osteoblasts. Therefore, in a way similar to other cytokines, IL-6 has potent effects on bone remodeling, with a capacity to induce osteoclastogenesis and local erosions.[16,31]

Most of these effects have been confirmed in animal arthritis models and in cultured human cells. The complexity of the actions of cytokines as a whole, as well as their synergies, antagonisms, and reciprocal induction, etc, as happens in vivo, are particularly difficult to interpret in the case of IL-6. The need for the IL-6R for its function, occasionally from other cell types, makes the direct translations of knowledge from ex vivo models to the reality of human physiopathology difficult. Therefore the best study model will be patient treatment with IL-6 antagonist tocilizumab, available in the near future.

Effects of IL-6 Antagonist (Tocilizumab) in RA

Tocilizumab is a monoclonal antibody for human use, capable of neutralizing the biologic effect of IL-6 through the specific blockade of its receptor IL-6R, which has been shown effective for the

treatment of RA. The impact of this antagonist on the systemic effects of inflammation and RA clinical aspects, as well as joint damage, allows us to propose that the mechanisms which have been stated here can explain the participation of IL-6 in the pathogenesis of RA and more or less occur to a certain degree.

Data available from clinical trials with tocilizumab indicate a rapid and potent reduction of the acute phase response, fever and anemia in RA or in different affections such as Castleman's disease or systemic juvenile chronic arthritis, in which these manifestations are very relevant.[37,38] Independent of the cause of inflammation, tocilizumab can block these systemic response, for example after surgery,[39] and this effect can have important implications in the diagnostic use of these manifestations as an infectious alarm.

In RA, available data show that after the administration of tocilizumab produces a rapid and profound descent CRP and variations in other biologic parameters dependent of IL-6, such as an increase in cholesterol and reduction in platelets and circulating neutrophils.[22,40]

Clinical improvement of RA patients treated with tocilizumab has been widely confirmed in several phase III trials, but from the physiological standpoint there is little data on the modification of immunological parameters and mediators after the administration of tocilizumab, and no studies exist in synovial fluid. We can only prove a significant reduction in RF after therapy, similar to what happens with others after improvement of disease.[40] An interesting piece of information is a descent in the basal levels of VEGF after the administration of tocilizumab in RA,[41] an effect which has also been shown after therapy with TNFα inhibitors.[42] VEGF is an important mediator in cell recruitment and synovial angiogenesis, and is induced in a synergic way in synoviocytes by TNFα and IL-6.

In conclusion, although there is no direct data on the effects of tocilizumab in the mechanisms of joint destruction, the first available data shows a preventive effect on the progression of radiological data in treated patients.[43] It would be interesting to evaluate in a deeper manner the effects of local and systemic markers of bone and cartilage metabolism in these patients to confirm the current hypothesis on the participation of IL-6 in the homeostasis of these tissues.

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