The New And Evolving Science Of IL-6 In Rheumatoid Arthritis

The Roles For IL-6 In Both Innate And Adaptive Immunity In RA

Leonard H. Calabrese, DO
Co-Editor

Professor of Medicine
Cleveland Clinic

Cleveland Clinic
Lerner College of Medicine
of Case Western Reserve University

Ernest Choy, MD
Co-Editor

Head of Rheumatology and
Translational Research

Cardiff University

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Dear Colleagues,

This is a very exciting time in the field of rheumatoid arthritis (RA). The more we understand about RA pathogenesis from basic and clinical research, the more equipped we are to understand this disease. We now know that cytokines play many key roles in the inflammation that drives RA. One such example is interleukin-6 (IL-6), a multifunctional cytokine that contributes to chronic inflammation in patients with RA.

Regeneron Pharmaceuticals and Sanofi Genzyme are excited to bring you additional educational material describing some of the fundamental immunology and clinical pathology we see in our RA patients through a series of scientific monographs entitled *The New and Evolving Science of IL-6 in Rheumatoid Arthritis*. In the first installment, we reviewed the signaling mechanisms of IL-6 that allow it to have widespread effects in RA. In the second installment, we reviewed the contributions of the IL-6 pathway to bone resorption in RA, and in the third installment we reviewed how persistently elevated IL-6 signaling may contribute to both articular and systemic manifestations of RA. Here we discuss the roles of IL-6 in both innate and adaptive immunity in RA.

We hope you find this latest installment informative and engaging.

Sincerely,

Leonard H. Calabrese, DO
Co-Editor

Professor of Medicine
Cleveland Clinic

Cleveland Clinic
Lerner College of Medicine
of Case Western Reserve University

Ernest Choy, MD
Co-Editor

Head of Rheumatology and
Translational Research

Cardiff University

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Introduction

Rheumatoid arthritis (RA) is characterized by activation of the innate and adaptive arms of the immune system, which mediate processes leading to autoimmunity, chronic inflammation, joint destruction, and systemic manifestations.[1] Activation of the immune system in RA is driven by a complex network of cytokines, including tumor necrosis factor-α (TNF-α), interleukins (IL)-1, 4, 6, 7, 12, 13, 15, 17, 18, and 23, and interferons (IFN).[1-4] In general, each of these individual cytokines exerts effects on a subset of immune cells, with differential roles in activating innate and immune responses. For example, cells of the innate immune system are highly responsive to TNF-α, and thus TNF-α has a prominent role in driving innate immunity.[1] IL-6 impacts virtually all cells of innate and adaptive immunity due to its versatile signaling mechanism.[5] Indeed, persistently elevated IL-6 signaling plays a key role in driving chronic inflammation by stimulating immunity, and orchestrating persisting interactions between the innate and adaptive immune systems.[3-5]

The quantitative contributions of the innate and adaptive immune systems to RA may vary for different patients. During the disease course of RA, considerable patient-to-patient variation exists in the number of affected joints, the levels of autoantibody titers and serum cytokines, the rate of joint destruction, and the response to treatment.[2,6] Consistent with this, the pathological mechanisms underlying RA are heterogeneous, and the existence of different disease subsets is evidenced by histological examination of synovial tissues.[7] A spectrum of cellular compositions has been unveiled in such samples, ranging from diffuse leukocyte infiltration to well-organized lymphocyte-containing follicle-like structures.[7] Another recent study described four major phenotypes of the RA synovium—lymphoid, myeloid, low inflammatory, and fibroid—each associated with distinct underlying gene expression signatures.[6] Expression of the IL-6 signaling components (IL-6, the IL-6 receptor [IL-6R], and glycoprotein 130 [gp130]) was observed across all phenotypes and was suggestive of a broad contribution of IL-6 to chronic inflammation across heterogeneous RA pathologies, and is consistent with a role for IL-6 in both innate and adaptive immunity.[5,6] The role of IL-6 in the innate and adaptive immune systems is the focus of this monograph, and will be discussed in more detail hereafter.

Overview of Innate and Adaptive Immunity

The innate and adaptive arms of the immune system have evolved to provide integrated and complementary functions for mounting an effective immune response **(Figure 1)**. Tissue injury produced by biologic, chemical, or physical insults is first "sensed" by cells of the innate immune system—macrophages, dendritic cells, neutrophils, other myeloid cells (eg, eosinophils, basophils, and mast cells), and natural killer cells.[8] The innate arm is particularly able to recognize conserved features of microbial pathogens, employing pattern-recognition receptors (PRR) such as Toll-like receptors (TLR), which detect conserved pathogen-associated molecular patterns (PAMP), including bacterial and fungal cell-wall components and viral RNA.[9] Detection of PAMPs by PRRs leads to the induction of inflammatory responses and innate host defenses, which include the phagocytosis and degradation of pathogens by antigen-presenting cells (APC; eg, macrophages and dendritic cells), and the release of proinflammatory cytokines into the extracellular space.[8,10] The innate response is considered nonspecific, but it is present at all times and is able to provide immediate protection against pathogens.[8]

In multicellular organisms, the intrinsic limitation conferred by the nonspecific nature of the initial innate response has led to the evolution and integration of additional specialized immune-competent cells, giving rise to adaptive immunity. The adaptive immune response allows for the generation of effector cells with specificity towards a particular pathogen or antigen.[8] However, induction of the adaptive response is dependent on the innate system, as antigens that are processed from phagocytosed targets are shuttled to the surface of innate APCs and presented to B and T cells, the primary effectors of the adaptive immune system.[8] Upon binding specific antigens presented by innate cells, T cells undergo expansion into either T helper (Th) cells or cytotoxic T (Tc) cells.[8] Th cells orchestrate and regulate immune responses by contributing to the extracellular cytokine/chemokine milieu, and by attracting additional innate and adaptive effectors.[8] Tc cells kill infected host cells through release of cytotoxins and by promoting apoptosis of the targeted cells.[8] B cells, following antigen recognition and upon receiving costimulatory signals from Th cells, differentiate into plasma cells and produce antibodies specific for the presented antigen.[8] B cells also shape T-cell maturation and effector T-cell responses through production of cytokines, including IL-6.[1] In addition, after a pathogen is cleared, a small number of T and B cells with specificity for certain antigens survive and remain in circulation. These so-called memory cells undergo rapid expansion when re-exposed to their target antigen, allowing for a more efficient immune response in case of reinfection.[8]

Adaptive Response

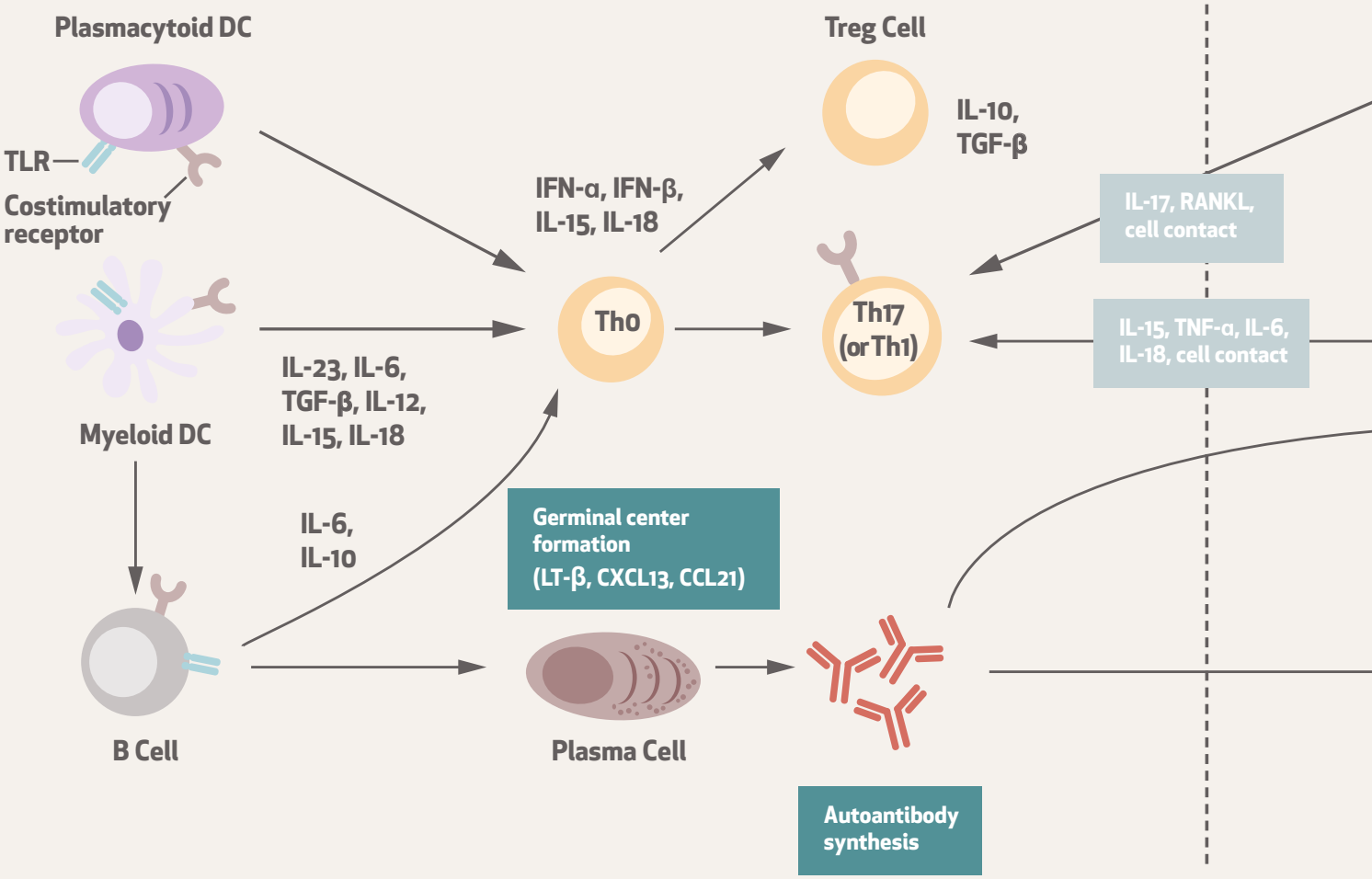
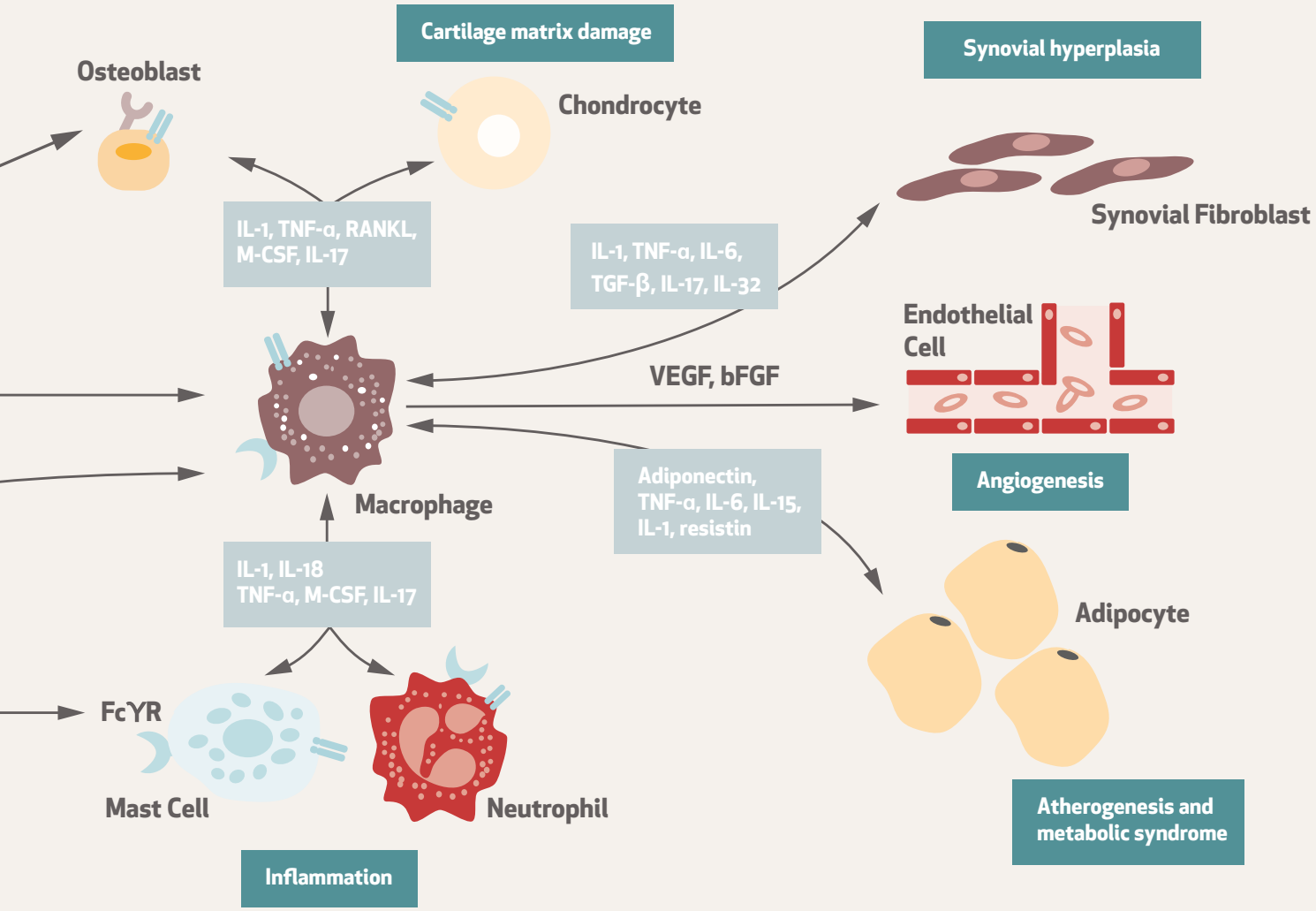


Figure 1. Innate and adaptive immune processes within the joint in RA. Dysregulated expression of cytokines drives activation of effector cells, which leads to the clinical manifestations of RA. bFGF, basic fibroblast growth factor; CCL21, chemokine (C-C motif) ligand 21; DC, dendritic cell; FcγR, Fcγ receptor; M-CSF, macrophage colony stimulating factor; LT-β, lymphotoxin-β; RANKL, receptor activator of NF-κB ligand; VEGF, vascular endothelial growth factor. Figure adapted with permission from McInnes et al. 2007.[4]

Innate Response



The activated innate and adaptive immune systems in the synovial membrane and adjacent bone marrow integrate to promote inflammation and tissue damage in RA.[11] The initial trigger for RA pathogenesis is unknown; however, it is thought that loss of tolerance to self-antigens is required for the induction phase of RA.[1] It has been suggested that APCs indiscriminately sample their surroundings and present endogenous ("self") antigens to T cells in the lymph nodes, which are incorrectly recognized as foreign, and the adaptive immune response is initiated.[1] Presentation of self-antigens by APCs cause T cells to undergo expansion and B cells to differentiate and produce autoantibodies such as rheumatoid factor (RF) or anti-citrullinated protein antibodies (ACPA).[1] Activated B and T cells also release cytokines, including IL-6, TNF-α, and IL-1, which further activate both innate and adaptive cells and also stimulate synovial stromal cells (eg, fibroblast-like synoviocytes [FLS] and chondrocytes) to synthesize cytokines and other soluble mediators conducive to an inflammatory milieu.[1] These signaling molecules recruit additional cell types to the inflamed joint, stimulate cellular proliferation, and help train adaptive immune cells (ie, B and T cells) to learn and memorize the target antigen.[1,3] Through establishment of positive feedback signaling loops and the continued presence of self-antigens, the innate and adaptive systems are always primed, and the inflammatory response continues unabated in RA.[4,12] Central to this process is IL-6, as persistently elevated IL-6 signaling instigates chronic inflammation by stimulating both innate and adaptive immunity, and facilitating their interaction.[3-5]

IL-6 and Innate Immunity

Cytokines modulate many facets of the innate immune response, inclusive of local and systemic effects, in part through their ability to act on innate effector cells.[5] Many of these cells—including macrophages, mast cells, and natural killer cells—are found in the synovial membrane, while others, such as neutrophils, reside mainly in the synovial fluid.[1] Macrophages are central effectors of synovitis in RA, promoting inflammation through their production and release of cytokines, reactive oxygen and nitrogen intermediates, prostanoids, and matrix-degrading enzymes.[1] Through cytokine signaling, macrophages can recruit and activate neutrophils and mast cells, which directly contribute to articular inflammation by synthesizing chemical mediators.[1,4] Activated effector cells of the innate immune system also synthesize additional cytokines that stimulate the cell types that are responsible for local structural damage in RA **(Figure 1)**.[1,4] For example, cytokines stimulate osteoclast activity, leading to increased bone resorption, and also activate chondrocytes and FLS to release matrix metalloproteinases (MMP)— enzymes which are responsible for cartilage degradation.[1] Activated FLS in RA also mediate synovial hyperplasia, leading to pannus formation.[1]

As described above, cytokines regulate many of the physiologic and pathologic functions mediated by the innate system. Chief among these is IL-6, which, along with TNF-α and IL-1, plays a prominent role in the innate immune

response.[5] Indeed, virtually all innate immune cells secrete, and are responsive to, IL-6.[5,13,14] While IL-6 is a key driver of local effects such as inflammation, it also enters the circulation to mediate the more systemic aspects of the innate response, including induction of the acute phase and febrile responses.[9,13]

IL-6 also influences innate immunity by orchestrating the interactions between stromal and effector cells of the innate immune system, which can lead to increased recruitment of immune cells to sites of infection or injury or, in the case of RA, to inflamed joints.[15] During acute inflammation, IL-6 is initially released by monocytes, macrophages, and endothelial cells; IL-6 signaling then mediates recruitment of neutrophils through activation of a subset of chemokines and adhesion molecules by endothelial cells, smooth muscle cells, epithelial cells, mesothelial cells, and FLS.[13] For example, in the presence of its soluble receptor (sIL-6R), IL-6 induces secretion of chemokine (C-X-C motif) ligand 8 (CXCL8) by FLS.[15] In turn, CXCL8 induces neutrophil chemotaxis and activation, and may contribute to the neoangiogenesis that is commonly found in inflamed synovia.[15-17] IL-6 has also been shown to prolong neutrophil survival through regulatory effects on neutrophil apoptosis, allowing for their extended contribution to articular inflammation.[18]

Importantly, IL-6 plays a key role in the transition from acute to chronic inflammation, through regulation of monocyte chemotaxis.[3,19] Proteases present in the synovial fluid enhance shedding of membrane-bound IL-6R from neutrophils, allowing stromal cells which do not express IL-6R to respond to IL-6 and to begin to produce monocyte-specific chemoattractants.[20-22] Another consequence of IL-6 signaling enabled by IL-6R shedding is a reinforcement of the interactions between innate and stromal cells, establishing a positive feedback loop, as demonstrated by in vitro cellular studies **(Figure 2)**.[15] In the synovia

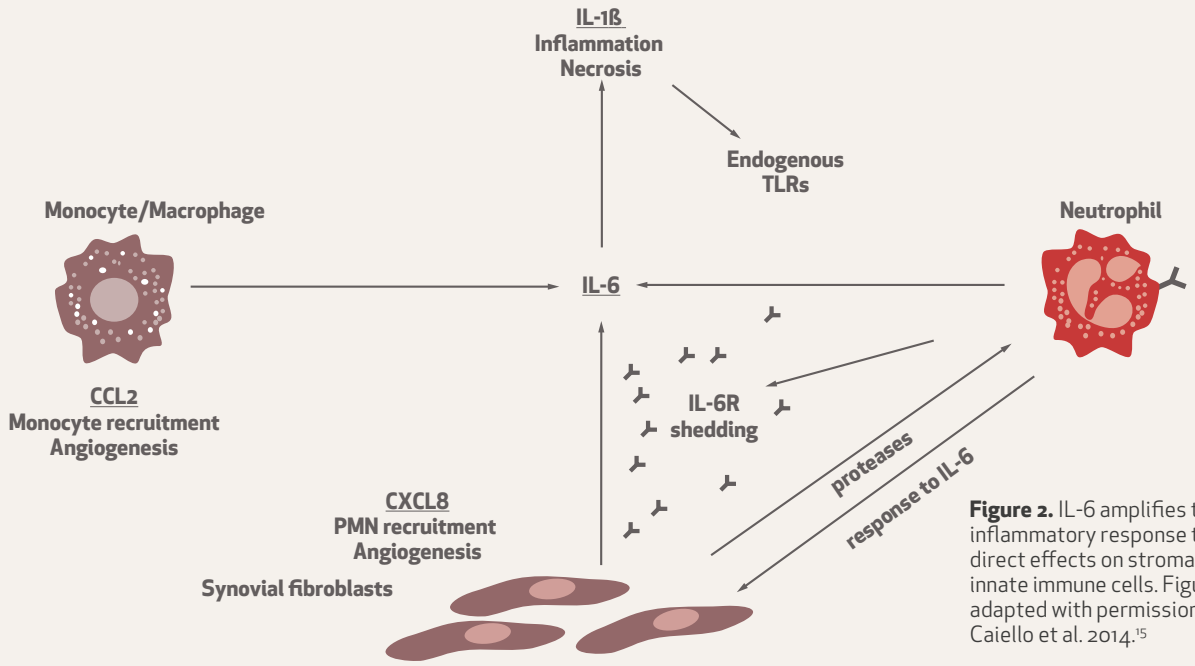


Figure 2. IL-6 amplifies the inflammatory response through direct effects on stromal and innate immune cells. Figure adapted with permission from Caiello et al. 2014.[15]

of RA patients with chronic inflammation, this positive feedback loop leads to, and is simultaneously caused by, persistently elevated cytokine signaling.[15]

Under normal conditions, circulating levels of IL-6 are maintained at low levels. Based on several reports, serum levels of circulating IL-6 in healthy subjects range from 1 to 16 pg/mL.[23-28] Conversely, in RA, serum IL-6 levels have been reported in the range of 5 pg/mL to 200 pg/mL,[24,25,27,29,30] with 100- to 1000-fold higher concentrations found in synovial fluid.[24,25,27,30-32] Consistent with this, IL-6 is one of the most abundant cytokines in the serum and synovial fluid of patients with RA and correlates with both disease activity and articular destruction.[3,31,33-35] In addition, elevated activity of signal transducer and activator of transcription 3 (STAT3)—one of the downstream transcription factors activated during IL-6 signaling—has been reported in the synovial tissue of mice induced with arthritis, and in vitro and in vivo studies have linked STAT3 to chronic disease progression through the regulation of synovial hyperplasia and proinflammatory mediators.[13,36,37]

IL-6 and Adaptive Immunity

Similar to the innate system, the various functions of T-cell–mediated and humoral (ie, antibody-mediated) immunity that constitute the adaptive immune response are modulated through cytokines.[1] Activated T and B cells, and their respective subtypes, are pivotal to the development of autoimmunity and drive RA disease progression through mechanisms including autoreactive antibody generation and the expansion of memory cells into effector cell populations with defined proinflammatory cytokine signatures **(Figure 1)**.[2] In T-cell–mediated immunity, activated synovial T cells contribute to synovitis directly through the production of inflammatory cytokines and via interactions with neighboring macrophages, FLS, and B cells that promote their activation.[1,5,8,38] In humoral immunity, activated B cells synthesize autoantibodies that include ACPAs, and IgM and IgG RFs, in the serum and in joints of patients with RA.[3] Besides serving as diagnostic markers of RA, these autoantibodies have been implicated in disease pathogenesis: patients with ACPA have more erosive RA and are less likely to stay in remission, and the presence of RF is associated with higher disease activity.[39-43]

As discussed above, macrophages and dendritic cells can influence adaptive immune responses through their function as APCs.

Innate cells can also exert effects on the adaptive system through cytokine signaling. Indeed, although TNF-α plays a central role in innate response, it can indirectly stimulate the adaptive system, by inducing innate cells to release cytokines like IL-6, which can directly affect both innate and adaptive immune cell types.[1] Consistent with this, IL-6–knockout mice are resistant to the development of antigen-induced arthritis, even though they express normal quantities of TNF-α and IL-1.[44,45] The differential effects of TNF-α and IL-6 on the innate and adaptive systems align with the functions ascribed to the downstream signaling systems that they activate.[2] When bound to its membrane-bound receptor (TNF-R1), TNF-α primarily activates the nuclear factor (NF)-κB pathway, which is also activated by binding of PAMPs to cells of the innate system.[2,46] IL-6 primarily activates the Janus kinase (JAK)/STAT pathway through its receptor complex, which, in contrast to the NF-κB pathway, is prevalent in both innate and adaptive systems.[2,47]

Humoral Immunity

As mentioned earlier, B cells, following antigen recognition, differentiate into plasma cells and produce antibodies specific for the presented antigen, which in the case of RA includes autoantibodies (ie, RF and ACPAs).[1] The involvement of IL-6 in antibody development has been firmly established, as IL-6 was originally identified as a T-cell–derived factor that induced the maturation of B cells into antibody-secreting cells and promoted the survival and maintenance of long-lived plasma cells **(Figure 3)**.[13,48] Besides inducing maturation, IL-6 also indirectly stimulates B-cell production by promoting the B-cell helper properties of $CD4^+$ T cells via the production of IL-21.[38,49,50] Consistent with these properties, animal models have shown that IL-6 deficiency is associated with diminished antibody response and susceptibility to infection.[51] More recent studies have demonstrated a role for IL-6 in the induction of another type of B cell referred to as regulatory B (Breg) cells, which—analogous to the function of Th cells—regulate B cells

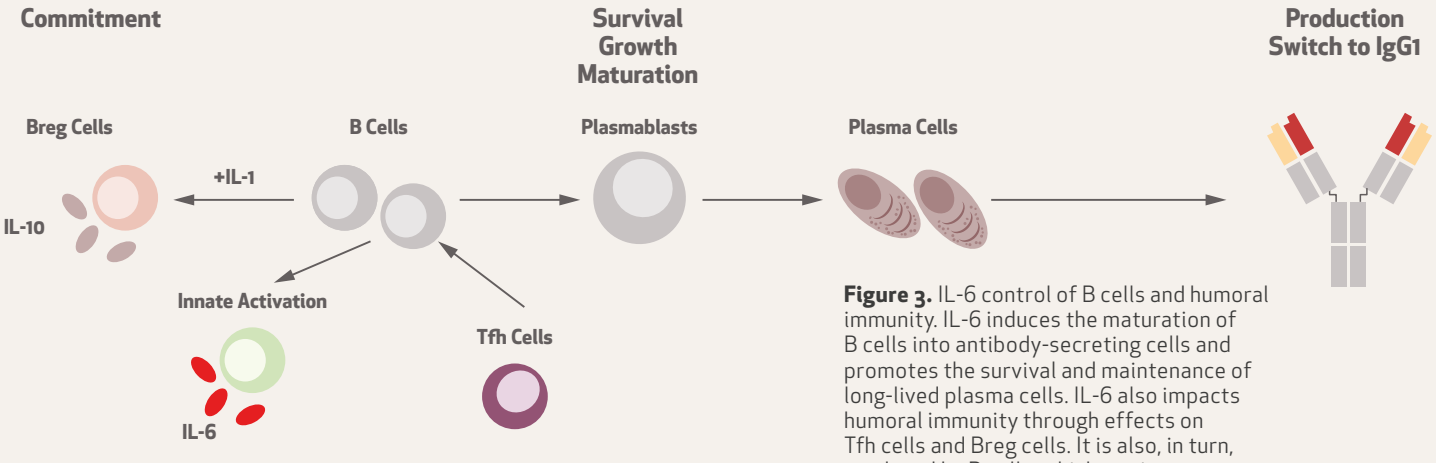


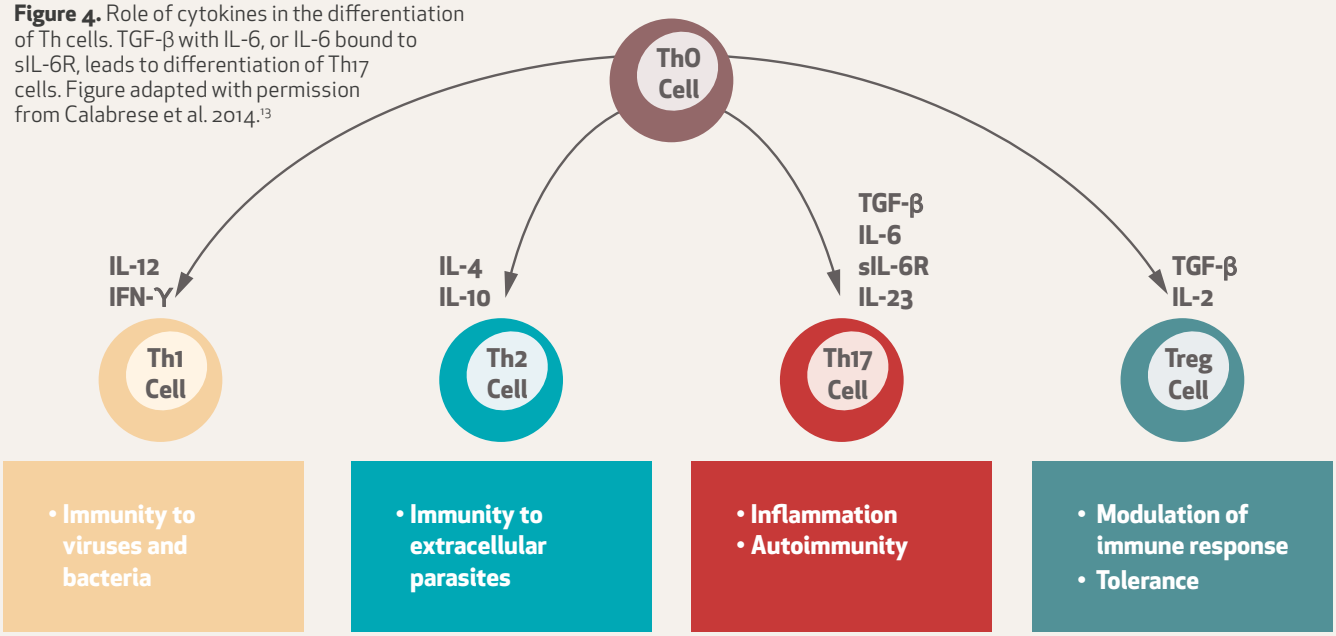
Figure 3. IL-6 control of B cells and humoral immunity. IL-6 induces the maturation of B cells into antibody-secreting cells and promotes the survival and maintenance of long-lived plasma cells. IL-6 also impacts humoral immunity through effects on Tfh cells and Breg cells. It is also, in turn, produced by B cells, which can impact effector cells of the innate response. Figure adapted with permission from Hunter et al. 2015.[5]

through cytokine signaling.[52-55] Yet another way in which IL-6 contributes to humoral immunity is through effects on follicular helper T cells (Tfh cells), a specialized subset of CD4+ T cells that express the chemokine receptor CXCR5 and localize to B-cell follicles where they promote B-cell proliferation and immunoglobulin class switching.[52,56] IL-6 also allows for the adaptive response to reciprocally impact the innate response, as activated mature B cells and regulatory B cells produce IL-6, which then triggers innate immune cells and stromal cells to produce cytokines, chemokines, and other proinflammatory factors.[5]

T-cell–mediated Immunity

IL-6 also plays a pivotal role in T-cell–mediated immunity, in part by specifying the subtype differentiation of Th cells.[13,57] For example, the presence of IL-6 specifically inhibits transforming growth factor (TGF)-β–induced differentiation of naïve T cells into regulatory T (Treg) cells—cells that inhibit T-cell activation and play a critical role in maintaining immune homeostasis and preventing autoimmune diseases.[13,57,58] Simultaneously, the combined presence of IL-6 and TGF-β drives specific differentiation of naïve T cells to the Th17 phenotype; consequently, IL-6–knockout mice are defective in generating Th17 cells **(Figure 4)**.[13,57-59] Th17 cells are key players in amplifying IL-6–mediated inflammation. IL-6 is produced by Th17 cells, further promoting Th17 differentiation,[60] and dysregulated responses of Th17 cells have been shown to contribute to local tissue damage in chronic inflammatory diseases.[61] In addition to IL-6, Th17 cells also produce IL-17, which has been implicated in the pathogenesis of RA, in part by facilitating bone resorption through increased osteoclast activity.[1,4] Exemplifying the role of amplified IL-6 expression during inflammation, IL-17 induces

Figure 4. Role of cytokines in the differentiation of Th cells. TGF-β with IL-6, or IL-6 bound to sIL-6R, leads to differentiation of Th17 cells. Figure adapted with permission from Calabrese et al. 2014.[13]



FLS to produce IL-6, further magnifying the IL-6–dependent effects of FLS.[60,62]

In stromal tissues, IL-6 *trans*-signaling (ie, signaling mediated by sIL-6R) regulates several inflammatory chemokines responsible for the recruitment of T cells.[5] Effector cytokines (eg, IL-17, IL-22, and IFN-γ), controlled by the action of IL-6 on CD4+ T cells, also have a direct bearing on the activities of innate immune cells and stromal tissues, which perpetuate inflammatory activation, promote immune cell retention, and shape the pathology observed within tissues.[5]

Conclusions

RA is a systemic disease driven by chronic joint inflammation that results in joint damage and loss of function.[3] Integrated activation of the innate and adaptive responses is a requisite for chronic inflammation, and is mediated by cytokine signaling. IL-6 is one such cytokine, and is able to interact with virtually any cell based on its versatile signaling mechanism.[3,63] Indeed, almost all cells of the innate and adaptive arms of the immune system, along with supporting stromal cells, respond to IL-6, and many of these cells also produce IL-6.[5] As a result, elevated IL-6 levels may contribute to persistently activated innate and adaptive immune systems responsible for RA disease chronicity and pathophysiology.

IL-6 facilitates not only communication among cells within the innate and adaptive immune arms, but also allows for interactions between the two. As discussed above, IL-6 produced by innate immune and supporting stromal cells can activate adaptive T and B effector cells. These effector cells also secrete IL-6, which reciprocally influences the function of cells of the innate system.[5,37] IL-6 also induces cells to produce other key cytokines that facilitate communication between the different cells of the immune system.[5] In this way, IL-6 can be viewed as a key messenger in autoimmunity and, in cases of persistent elevation like RA, IL-6 contributes to a state of chronic inflammation. Continued research on the many functions of IL-6 may further delineate the pathological origins and underpinnings of RA.

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