Review Article

Pathogenesis of Collagen-Induced Arthritis: Role of Immune Cells with Associated Cytokines and Antibodies, Comparison with Rheumatoid Arthritis

(collagen-induced arthritis / CIA / rheumatoid arthritis / animal model / immunopathology / cytokines / antibodies, interleukin)

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Abstract. Collagen-induced arthritis is the most common *in vivo* model of rheumatoid arthritis used for investigation of new potential therapies in preclinical research. Rheumatoid arthritis is a systemic inflammatory and autoimmune disease affecting joints, accompanied by significant extra-articular symptoms. The pathogenesis of rheumatoid arthritis and collagen-induced arthritis involves a so far properly unexplored network of immune cells, cytokines, antibodies and other factors. These agents trigger the autoimmune response leading to polyarthritis with cell infiltration, bone and cartilage degeneration and synovial cell proliferation. Our review covers the knowledge about cytokines present in the rat collagen-induced arthritis model and the factors affecting them. In addition, we provide a comparison with rheumatoid arthritis and a description of their important effects on the development of both diseases. We discuss the crucial roles of various immune cells (subtypes of T and B lymphocytes, dendritic cells, monocytes, macrophages), fibroblast-like synoviocytes, and their related cytokines (TNF-α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-17, IL-23, GM-CSF, TGF-β). Finally, we also focus on key antibodies (rheumatoid factor, anti-citrullinated protein antibodies, anti-collagen II antibodies) and tissue-degrading enzymes (matrix metalloproteinases).

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease of joints that is typically accompanied by significant extra-articular symptoms. Characteristic manifestations of this autoimmune imbalance include cartilage deformation and bone erosion leading to severe disability. Despite the huge progress in the field of pharmacology, the overall therapeutic success in RA is not fully satisfactory. The current therapy includes conventional synthetic disease-modifying anti-rheumatic drugs (DMARDs) and biological DMARDS; both in a potential combination with glucocorticoids. Chronic administration of conventional synthetic DMARDs is associated with significant side effects. Biological DMARDs are related to a risk of developing resistance and burden the healthcare system with high financial requirements (Smolen et al., 2016; Lin et al., 2020). The exploration of new options for complex treatment of RA is therefore a therapeutic challenge.

To investigate new potential therapies in preclinical research of RA, animal models are fundamental. Generally, the most popular are smaller animal species (mice, rats, rabbits, guinea pigs) because of the length of their life cycle, shorter disease onset than in humans, relatively low intra-individual variability, low price and easy manipulation. Contemporary models of RA comprise two different approaches – induced animal models (A) and genetically manipulated models (B). The first group serves as a tool for investigation of the role of immune

Received July 7, 2023. Accepted October 11, 2023.

This study was supported by AZV grant No. AZV NU22-08-00346, Charles University Cooperatio programme (research area PHAR) and Charles University grant No. SVV 260638.

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Abbreviations: Ab – antibodies, anti-CII Ab – anti-collagen II antibodies, CIA – collagen-induced arthritis, DC – dendritic cells, DMARDs – disease-modifying anti-rheumatic drugs, FLS – fibroblast-like synoviocytes, GPSM2 – G-protein-signalling modulator 2, IC – immune complexes, MMPs – metalloproteinases, NK – natural killer cells, RA – rheumatoid arthritis, RF – rheumatoid factor, ROS – reactive oxygen species, STAT 3 – signal transducer and activator of transcription, TCR – T-cell receptor, TLR – Toll-like receptor, T reg – regulatory T cells, VASP – vasodilator-stimulated phosphoprotein.

cells and chemokines in the process of this systemic autoimmune disease. It can also be used for observation of the sequential development of inflammation (manifestation of redness, swelling, heat, pain → joint impairment). (A) includes the adjuvant-induced arthritis model, pristane-induced arthritis model, streptococcal cell wall-induced arthritis model, cartilage oligomeric matrix protein-induced arthritis model, and antigen-induced arthritis model. Presently, the most frequently used type is the collagen-induced arthritis model (CIA), which is considered as the gold standard of *in vivo* models. Genetically manipulated models are narrowly directed to the role of genes in the spontaneous onset and development of RA. The second group (B) includes, for example, the K/BxN and tumour necrosis factor (TNF)-α transgenic mouse model (Li and Schwarz, 2003; Monach et al., 2008). It is important to mention that none of these experimental models can entirely mimic the conditions of the human body, but there is no doubt about their contribution to the RA research (Tanushree and Saikat, 2013; Choudhary et al., 2018; Ye et al., 2021). In this review, we would like to focus on CIA as one of the most extensively studied standard models of RA (22 thousand results on PubMed).

The development of RA and the severity of arthritis in animal models are evaluated by various methods: imaging observation (X-ray, micro-CT) (Hu et al., 2017), histological analysis (Zhang et al., 2009), clinical score (assessment of paw thickness, redness, swelling), and serum analysis (Chondrex, 2015). Because of the possible variability in the assessment of histology and radiological outcomes, the best option for comparison of each experiment is to evaluate the biochemical markers (antibodies, cytokines).

Cytokines are implicated in each phase of the pathogenesis of RA and CIA from the pre-articular phase to promoting the autoimmune process and to joint destruction. At present, the cytokine targets of biological and targeted synthetic DMARDs include interleukin IL-1, IL-6, IL-12/23, IL-17, TNF-α inhibitors, and granulocyte-macrophage colony-stimulating factor inhibitors. The modern therapy approaches result in a rapid and preserved improvement of clinical signs and symptoms, along with reduced radiological progression of joint destruction. Nonetheless, the complete remission of RA is still an unattainable goal (Radu and Bungau, 2021).

The aim of this review is to characterize the cytokines present in the rat CIA model and the factors affecting them, to compare them with RA, and to describe their important role in the development of both diseases.

Literature search

We searched PubMed and Web of Science databases up to March 2023. The medical subject headings were "collagen-induced arthritis", "immunopathogenesis", "immune response", "T lymphocytes", including "Th1, Th2, Th17, Th reg", "B lymphocytes" and "dendritic cells". These terms were combined with the designations of cytokines "IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, IL-23," other factors "TNF-α, GM-CSF, INF-γ, TGF-β" and antibodies "RF, ACPA, anti-CII Ab" to find relevant references. Target keywords were searched in the titles and abstracts. Results were limited to publications written in English and focused on the CIA rodent model. A total of 3,322 results were found in the literature search, out of which 70 publications were relevant to our review.

Comparison of CIA and RA

CIA was first reported by David Trentham et al. (1977). As was mentioned above, CIA is considered to be the gold standard in research, because it shows many similarities to human RA (comparison is summarized in Table 1). The employed rats and mice exhibit the pathological features of RA, including redness, swelling and pain caused by synovitis and polyarthritis, together with joint and bone malformations (pannus formation, bone and cartilage erosion, and fibrosis) (Trentham et al., 1977; Luross and Williams, 2001; Brand et al., 2003; Poutoglidou et al., 2020). Systemic (extra-articular) manifestations were also observed – secondary osteoporosis (Wu et al., 2016), diastolic dysfunction, and others.

Even though CIA is an optimal approach to investigating new therapeutic agents for RA, it is important to note that one gene-analysing report describes significant differences in the gene expression in human and rat arthritic tissues (Soto et al., 2008). Notably, the most up-regulated genes in both these diseases were metalloproteinases (MMPs), suggesting active extracellular matrix remodelling. In contrast to CIA (MMP-3, MMP-13, MMP-14), in human RA, lower amounts of up-regulated genes of this type (MMP-1) were present, but more genes associated with immunoglobulins (related to the presence of plasma cells) and chemokines (CXCL13, CXCR4) (related with an influx of B, T lymphocytes and macrophages into the synovial tissue) were induced. However, in CIA, chemokine genes were predominant (CCL9, CCL20, CCL7), which are chemotactic for monocytes, macrophages, neutrophils, immature dendritic cells, and weakly for T lymphocytes. Up-regulation of genes associated with B lymphocytes and plasmatic cells was not detected in the CIA lesions. In conclusion, the data of this report showed a large presence of B lymphocytes and related gene expression in samples of RA arthritis tissue, whereas in the case of CIA, is appeared that the predominant cell types were of myeloid origin – macrophages or dendritic cells. B lymphocytes play a significant role in the RA pathogenesis, and their absence in the CIA model suggests that CIA would not accurately represent the humoral immune system of RA.

The CIA autoimmune model should be induced in genetically susceptible strains of rats or mice, because the expression of specific major histocompatibility complex (MHC) class II genes is a crucial condition for the onset of the disease (Luross and Williams, 2001; Chondrex, 2015). In addition, based on the research findings it is evident that CIA is determined by not only MHC genes but also by a huge complex array of other impor-

Table 1. Comparison of the main features of CIA and RA (Brand et al., 2007; McInnes and Schett, 2007; Rowley et al., 2008; Schurgers et al., 2011; Forster et al., 2012; Song et al., 2015; Wu et al., 2016; Kondo et al., 2021)

Human RA	**CIA model**
Similarities:	
Susceptibility to RA is associated with specific MHC-II haplotype (HLA-DRB1) and other genes (PTPN22, PADI4, CTLA4…)	Predisposition to CIA is associated with specific MHC-II haplotype (H2 complex in mice, RT1 locus in rats)
Incidence higher in females	Incidence higher in female Wistar rats
Polyarthritis	Polyarthritis
Redness, swelling, pain	Redness, swelling, pain
Joint and bone malformations (pannus formation, erosion of the bone and cartilage, fibrosis)	Joint and bone malformations (pannus formation, erosion of the bone and cartilage, fibrosis)
Systemic (extra-articular) manifestation	Secondary osteoporosis, diastolic dysfunction
High articular levels of various pro-inflammatory cytokines	High articular levels of various pro-inflammatory cytokines
Induction of RANKL on osteoblastic cells leading to osteoclastogenesis → bone destruction caused by osteoclasts	Induction of RANKL on osteoblastic cells leading to osteoclastogenesis → bone destruction caused by osteoclasts
Markers in serum: Rheumatoid factor Anti-CII antibodies Anticyclic citrullinated peptide antibodies	Markers in serum: Rheumatoid factor Anti-CII antibodies Anticyclic citrullinated peptide antibodies
Differences:	
Chronic	Self-limiting
Significant differences in gene expression in arthritic tissue – strong presence of B lymphocytes in RA arthritic tissue	Significant differences in gene expression in arthritic tissue – predominant presence of myeloid cells (macrophages, dendritic cells) in CIA arthritic tissue
Rheumatoid nodules	Absent
Morning stiffness	Absent

tant genes (Griffiths and Remmers, 2001; Gwon et al., 2018). This is the reason for the different susceptibility among the animal strains. Previous research has shown that rat strains are more susceptible to CIA than mouse strains, and that Wistar rats are the most responsive. In addition, female Wistar rats exhibited the highest incidence (83.3 %) and severity of the disease (Song et al., 2015).

Immunization of animals is made by subcutaneous injection into the bases of adult rats' tails with highly purified type II collagen, which is present as a major component in the joint cartilage. It is possible to use various types of collagen II – porcine, bovine, chicken, or rat (Chondrex, 2015). Moreover, to achieve the best arthritis incidence, it is recommended to prepare an emulsion of collagen II with complete or incomplete Freund's adjuvant (mineral oil with or without heat-killed *M. tuberculosis*). Immunization provokes production of specific collagen-II antibodies, which attack the joint tissue. The onset of polyarthritis in female Wistar rats generally occurs around 10 to 17 days post injection (Holmdahl et al., 2002; Brand et al., 2007; Jelínek et al., 2022).

Role of immune cells and associated cytokines in CIA and RA

It is currently known that the pathogenesis of CIA and RA mainly affects synovial joints, where immune cells (T and B lymphocytes, macrophages, and dendritic cells) infiltrate the synovium. Additionally, fibroblast-like synoviocytes (FLS) present in the sublining layer of the synovium proliferate, create the pannus and contribute to cartilage damage.

The aforementioned cells play a crucial role in the pathogenesis of CIA as well RA, but the exact mechanism is unknown. The molecule of collagen II is post-translationally modified, and these modifications may be recognized by dendritic cells (DC) (Holmdahl et al., 2002). Subsequently, activated DC migrate to draining lymph nodes, where they present the particular CII peptide by MHC class II to naïve T cells. This cell communication increases the occurrence of specific DC subpopulations in mice ($CD8\alpha^+$) and stimulates production of pro-inflammatory cytokines such as TNF-α, IL-2, IL-5, IL-6, IL-12, IL-17, and IL-23 (Holmdahl et al., 2002; Leung et al., 2002; Cho et al., 2007). DC have an elementary function in regulating immune responses by taking up, processing and presenting antigens to naïve T cells. Interestingly, it has been shown that some types of DC play a role in suppressing the autoimmune response and some types are required for the initiation of CIA (Leung et al., 2002; Jongbloed et al., 2009; Ramos et al., 2020). In RA patients, enhanced migration of the "inflammatory" $CD14^+CD1a^+CD1c^+$ subtype of DC to the inflamed joints was reported along with production of

TNF-α, TGF-β, IL-1β, IL-6, and mainly IL-12 and IL-13, leading to Th17 activation that resulted in imbalances between Th1, Th2 and Th17 lymphocytes, with final development of inflammation (Segura et al., 2013; Lin et al., 2020).

Enlarged spleen and lymph nodes draining the hind paws of the CIA rat model indicate that increased cell proliferation contributed to the initiation of inflammation. Additionally, a significant increase in the number of B lymphocytes in the blood and lymph nodes was observed in CIA animals. Further, the presence of myeloid cells (CD11b/c$^+$) was significantly higher in the blood when compared to the control. Interestingly, the amount of T lymphocytes was significantly decreased in lymph nodes, whereas natural killer cells (NK) were increased in the spleen (Teixeira et al., 2019). This report also showed that the proportions of CD8$^+$ and CD4$^+$ T cells were similar between all animals in the blood and spleen; however, the presence of CD4$^+$ T lymphocytes was decreased in the lymph nodes compared to the control (Teixeira et al., 2019). Various leukocytes migrated to the inflamed ankle tissue and increased expression of adhesive molecules and chemokines, which progressed into intensified production of chemokines and pro-inflammatory and anti-inflammatory cytokines (Nevius et al., 2016).

Joint inflammation mediated by T lymphocytes and their cytokines

Bibliographic sources report that one of the major players in the pathogenesis of CIA and RA are T lymphocytes, especially CD4$^+$ T cells (and their subsets Th1, Th17, T reg) and CD8$^+$ T cells (Alzabin and Williams, 2011). Unfortunately, the accurate molecular mechanism is perplexing. The consideration that MHC-restricted CD4$^+$ T cells are an important component is based on the fact that the human HLA DRB1 genotype is a crucial genetic risk factor for the development of RA (and the susceptibility to CIA is also associated with a specific MHC-II haplotype) (Alzabin and Williams, 2011; Sumitomo et al., 2018). CD4$^+$ T cells were detected in synovial tissue of the CIA model, and genetically modified mice with CD4$^+$ T cell depletion were less susceptible to the disease, but not completely resistant (Ehinger et al., 2001; Kang et al., 2021). In a recent report (Kang et al., 2021), authors describe that infiltrated CD4$^+$ T cells in synovial tissue have up-regulated specific zinc transporter (ZIP8) in samples from the CIA model and RA patients compared to the control. The deficiency of this ZIP8 transporter substantially decreases the influx of zinc, which leads to abrogation of the T-cell receptor (TCR)-mediated signalling pathway, attenuating the function of effector CD4$^+$ T cells and Th17 cells. Moreover, a significant reduction in IL-17A, TNF-α, IL-2 levels and augmentation of IL-10 were observed in ZIP8-deficient mice.

Th1 cells were one of the first T-helper cell subsets described in the pathogenesis of RA and CIA, implicating RA and CIA as Th1-dependent diseases. By secreting pro-inflammatory cytokines (IL-2, IFN-γ and TNF-β), Th1 lymphocytes communicate via a signalling network with other immune cells, resulting in activation of macrophages and B cells and consequently in the cell-mediated inflammatory response. INF-γ stimulates local antigen-presenting cells to produce IL-12, leading to the differentiation of CD4$^+$ T cells into INF-γ-secreting Th1 cells. However, animal models lacking the gene for INF-γ, INF-γ receptor, or treatment by INF-γ were not protected against CIA development; by contrast, CIA was more severe in such cases (Alzabin and Williams, 2011; Schurgers et al., 2011).

Moreover, INF-γ is missing or presented at low levels in the synovium of RA patients and it is rarely detectable in the synovial fluid (McInnes and Schett, 2007). At present, IL-17, produced by Th17 cells (a subset of CD4$^+$ T cells), is considered the key pathogenic cytokine, and it has been proposed that INF-γ suppresses inflammation through inhibition of Th17 responses (Lee et al., 2013).

Contrary to these findings, marker CXCR3 was identified on the surface of Th1 lymphocytes, and around 40 % of citrulline-reactive CD4$^+$ T cells were found to be CXCR3 positive in peripheral blood samples of RA patients. These data indicate a Th1 signature of autoreactive T cells in RA (James et al., 2014; Chemin et al., 2019). Transfer of citrulline-reactive CD4$^+$ T cells into CIA mice caused amplification of the severity of arthritis with increasing circulation of IgG2a anti-mouse CII antibody levels and synovial citrulline-reactive CD4$^+$ T-cell infiltration. It has been suggested that Th1 cells influence class switching toward IgG1 and IgG3 in humans. This idea corresponds with increased circulation of IgG2a anti-mouse CII antibody levels in the CIA model (Cordova et al., 2013).

The development of Th17 lymphocytes requires cooperation of IL-6, TGF-β, IL-21 and IL-23 signalling with TCR stimulation, followed by required retinoid orphan receptor γt (RORγt) receptor expression via transcription factor signal transducer and activator of transcription 3 (STAT3) activation (Barbi et al., 2013). The term IL-17 covers a family of cytokines composed of IL-17 A to F, where each interleukin plays a small distinct role in the pathogenesis of CIA and RA. In the study evaluating IL-17 in the CIA model (Yamaguchi et al., 2007), CD4$^+$ T cells isolated from the arthritic tissue expressed IL-17A and IL-17F significantly; IL-17B was expressed only in inflamed cartilage and IL-17C was expressed not only by CD4$^+$ T cells but also by CD11b$^+$ MHC II macrophages and CD11c$^+$ MHC II dendritic cells. Moreover, IL-17A and F stimulate production of inflammatory cytokines in fibroblasts (IL-6), and IL-17B, C boost production in macrophages (TNF-α and IL-1β). Notably, adoptive transfer of CD4$^+$ T cells over-expressing IL-17B and IL-17C exacerbated the progression of CIA. Finally, treatment of CIA mice by anti-mouse IL-17B antibodies significantly suppressed the progression of CIA in comparison with a vehicle, and a reduc-

tion of cell influx to the inflamed tissue and pannus was observed. In agreement with this, the pivotal role of IL-17 cytokines is also confirmed by studies focused on RA. Increased levels of IL-17 homologues were found in the serum and synovial fluid of RA patients compared to the control group and patients suffering from osteoarthritis (Kotake et al., 1999; Kim and Hwang, 2005).

Data from animal models indicated that Th17 and IL-17 can enhance pannus growth by accelerating mitochondrial dysfunction and inhibiting apoptosis of FLS by induction of their autophagy subsequently (Ito et al., 2011; Kim et al., 2017). FLS are a major cell population of the pannus and contribute to cartilage damage. Furthermore, IL-17 facilitates release of pro-inflammatory cytokines and chemotactic factors by monocytes, macrophages, or FLS (TNF-α, IL-1β, IL-6, IL-8, IL-16) (Yamaguchi et al., 2007; Cho et al., 2008; Ito et al., 2011). IL-17 also supports infiltration of inflammatory cells, increases production of VEGF important for angiogenesis (Ryu et al., 2006) and facilitates bone destruction through IL-17-mediated induction of RANKL on osteoblastic cells leading to osteoclastogenesis (Sato et al., 2006). Besides, IL-17 might also increase the expression of MMP-13 in synovial tissue of the CIA model, because injection of IL-17A neutralizing antibody into rat joint cavity significantly decreased MMP-13 expression (Shui et al., 2017). Finally, it is important to mention that the main factors suppressing the levels of IL-17 are INF-γ (Th1 cytokine) and IL-4 (Th2 cytokine) (Sarkar et al., 2009).

With continued research, a typical feature of RA was discovered – an imbalance of Th17 and T regulatory cells (T reg or also $CD4^+CD25^+Foxp3^+$ cells) favourable to pro-inflammatory Th17 cells. It is known that T reg cells have an essential role in the maintenance of self-tolerance and immune homeostasis by influencing $CD4^+$, $CD8^+$ T cells, NK cells, B lymphocytes, monocytes and dendritic cells. Their suppressive effect is attached to the production of IL-2, IL-10, and TGF-β as well as expression of cell surface molecules (CTLA-4, CD25, TIGIT) and intracellular molecules (granzymes, cAMP) (Fan et al., 2018; Kotschenreuther et al., 2022). T reg cells play an opposite role to Th17, because they can suppress the activation and proliferation of $CD4^+$ T lymphocytes, and their depletion activates spontaneous development of autoimmune diseases and intensifies the severity of symptoms of the CIA model (Jaen et al., 2009). Interestingly, the higher progression of the disease was not associated with augmented anti-collagen II antibody production. Another study reports that a single transfer of T reg cells slowed CIA progression (and also impaired the levels of IL-6 and TNF-α) and suppressed bone erosion, but the production of anti-collagen II antibodies was not reduced (Kelchtermans et al., 2009).

Among the main factors that modify the balance of Th17-T reg cells are TGF-β, cytokines, glycolysis and insulin-like growth factor 1 receptor. T reg cells found in RA patients are characterized by impaired functions and by an altered phenotype with modified production of proteins regulating T-cell migration – vasodilator-stimulated phosphoprotein (VASP) and G-protein-signalling modulator 2 (GPSM2) (Kotschenreuther et al., 2022). Medication aimed to influence the Th17-T reg balance already exists (IL-6 receptor inhibitor Tocilizumab), but the therapy is associated with severe adverse effects because of the interference with immune homeostasis.

At the end of this section, we cannot forget to mention the Th1/Th2 imbalance with predominant activity of Th1 cells in the synovial fluid and serum of RA patients. Nevertheless, a superiority of Th2 cytokines (IL-4, IL-13) was detected in synovial fluid of early-stage RA patients, which disappeared when RA was fully established (Raza et al., 2005). This implies that these cytokines are involved in the early modulatory response of the immune system. It has been suggested that by secretion of cytokines (IL-4, IL-10), Th2 lymphocytes have an anti-inflammatory effect on rheumatoid joint tissue, where they attenuate the production of IL-1β and TNF by synovial macrophages. Studies using the CIA model have also described a protective role of IL-4, which was boosted by IL-10 (Alzabin and Williams, 2011).

Contribution of B cells and their antibodies to the pathogenesis of RA

Aside from the large T lymphocyte roles discussed above, the presence of B lymphocytes and their response comprising anti-CII antibody (Ab) production is essential for the development of CIA, because T-cell recognition of peptides of collagen II (CII) alone is insufficient. B cells serve as antigen-presenting cells to activate antigen-specific T cells. These roles are supported by the observation that mice lacking mature B cell were fully protected against CIA and had a severely impaired ability to mount isotype-switched humoral immune responses against CII. Moreover, mice unable to form the germinal centre (microstructure in inflamed arthritic tissue formed from memory B cells and plasma cells) releasing Ab were also resistant to the CIA development (Dahdah et al., 2018). In addition, B cells can contribute to the pathogenesis of RA and CIA by producing cytokines such as IL-6.

Antibodies recognizing CII (mainly IgG1, IgG2a, IgG2b) are considered to be the key pathogenic factors in CIA, because they can bind and destabilize cartilage. Interestingly, epitopes of CII peptides recognized by antibodies and by T cells in murine models are coincident with those identified in human RA (Rowley et al., 2008). Anti-CII Ab can form local immune complexes (IC) that can set off synovial cell activation and later neutrophil infiltration of the articular tissue. Notably, these circulating IC appear in blood circulation around the term of the first clinical signs of CIA in the rat model. Subsequently, anti-CII Ab and these IC can trigger a complement system on the articular cartilage surface, with C5a appearing to be the most potent inducer of inflammation (Holers and Banda, 2018).

Rheumatoid factor (RF), the main validated marker of the disease severity in humans, also occurs in the rat and mouse CIA models. RF consists of autoantibodies against the Fc portion of IgG antibodies. Its higher levels can relate to more severe disease in CIA (Beduleva and Menshikov, 2010; Haikal et al., 2019), but also may not (Forster et al., 2012). In addition, the levels of RF in animal models seem to be in linear relationship with the levels of the specific types of anti-CII Ab (Rowley et al., 2008). However, the exact role of RF is still unknown, because RF is also detectable in healthy persons and there is a group of RA patients (20 %) who are RF seronegative (Radu and Bungau, 2021). Beside that, a major part of the original articles focused on CIA do not mention RF, but it is not possible to say whether the RF was not in the focus of the authors or whether RF was undetectable.

The production and accumulation of antibodies is detected in the CIA model before the initiation of clinical signs. This is similar to other autoimmune diseases including RA, where the presence of circulating antibodies years before the clinical stage is also observed. The first detectable Ab in the serum and synovial fluid of patients with RA are RFs, followed by anti-citrullinated protein antibodies (ACPA) and after that, anti-CII Ab during the disease onset (Dahdah et al., 2018; Holers and Banda, 2018; Lin et al., 2020). Comparable to RA, ACPA are also elevated in animal models (Forster et al., 2012).

The amount of ACPA is associated with an increased risk of developing bone erosion in patients suffering from RA. The formed immune complexes (ACPA with citrullinated proteins) activate macrophages by Fc receptors on their surface and induce the release of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-8 promoting the differentiation of osteoclasts (Coutant, 2019). In the CIA model, immune complexes can be bound on various subtypes of IgG receptors (FcγRI, FcγRIIb, FcγRIII and FcγRIV) expressed on a broad variety of immune cells (neutrophils, macrophages, NK cells and dendritic cells) with activation of secretion of cytokines (TNF-α, IL-1β, IL-8, IL-10, IL-12, IL-17, IL-23) and chemokines (CXCL13, MIP-1, MIP-2) supporting cell infiltration. The increased levels of pro-inflammatory cytokines affect synoviocytes, chondrocytes, synovial fibroblasts and macrophages in articular tissue and trigger the release of many other cytokines, most prominently IL-1β, TNF-α, IL-6, IL-8, IL-17, IL-23, GM-CSF, and tissue-degrading enzymes, with resulting severe inflammation and tissue damage (Rowley et al., 2008; Szarka et al., 2012; Kondo et al., 2021).

One of the most important mediators of joint inflammation is TNF-α produced by infiltrating T and B lymphocytes, synovial macrophages and NK cells. Its expression is increased in the arthritic tissue in RA and CIA, and it is known that over-expression of TNF induces spontaneous autoimmune arthritis in transgenic animal models.

It is currently known that TNF-α is involved in many steps of the pathogenesis of CIA and RA: TNF-α induces cartilage degradation and bone resorption, enhances osteoclastogenesis and increases production of other inflammatory cytokines (mainly IL-1β, IL-6 and TNF itself) by activating endothelial cells, synovial fibroblasts and macrophages (Lin et al., 2020; Kondo et al., 2021; Nam et al., 2022). Moreover, TNF-α controls the development of Th1 and Th17 lymphocytes and antibody production, interferes with the regulation of cell apoptosis and mitophagy, and raises production of reactive oxygen species (ROS). Presently, two TNF receptor isoforms are known: TNFR1 and TNFR2. Activation of TNFR1 signalling pathways promotes the pathogenesis of arthritis, while signalling via TNFR2 exerts protective functions in the CIA model (Kondo et al., 2021).

IL-6 (produced by endothelial cells, fibroblasts, monocytes, T and B lymphocytes, and synovial fibroblasts) also plays an important role in both innate and acquired immunity. IL-6 triggers production of acute phase proteins (e.g. CRP, SAA, fibrinogen), supports immune cell differentiation and migration (neutrophils, mononuclear cells) and influences T and B cells (towards increased secretion of Ig Ab by plasma cells) (Pandolfi et al., 2020). Furthermore, IL-6 production is associated with stimuli such as Toll-like receptor (TLR) ligands, IL-1β and TNF-α. Moreover, experiments with the genetic knockout mice model indicate that IL-6 induces differentiation of Th17 lymphocytes, and IL-17A triggers the positive-feedback loop of IL-6 expression through activation of transcription factors nuclear factor kappa light chain enhancer of activated B cells (NF-κB) and STAT3 in fibroblast cells. In depth, blockage of this loop significantly suppresses progression of arthritis in the mouse models. We can conclude from these reports that TNF-α and IL-17 synergistically activate synovial fibroblasts to produce IL-6 and that coordinated collaboration between TNF-α, IL-17 and IL-6 is required in the development of RA (Ogura et al., 2008; Zrioual et al., 2009; Slowikowski et al., 2020; Kondo et al., 2021).

Finally, results based on significant reduction of RA symptoms by a recombinant human IL-1R antagonist (Anakinra) showed that another indispensable pro-inflammatory cytokine in RA pathogenesis is IL-1. IL-1β, a member of the IL-1 family, largely produced by monocytes and macrophages, enhances inflammation by involvement in many signalling pathways. IL-1β simultaneously activates monocytes and macrophages, resulting in enhancement of IL-1β and TNF secretion (Kondo et al., 2021). IL-1β also induces proliferation of FLS, which are the main source of IL-6 in the intimal lining of the synovial membrane, and tissue-degrading enzymes such as MMPs, cathepsins and mast cell proteases, leading to cartilage and bone damage (Yoshitomi, 2019). IL-1β promotes development (via activation of RANKL) of osteoclasts leading to bone resorption and affects T reg cells to become osteoclastogenic T reg accelerating bone erosion (Nakamura and Jimi, 2006; Levescot et al., 2021).

Cartilage degeneration and bone erosion

To summarize, the coordination of IL-1β, IL-6, TNF-α and IL-17 may amplify the effect of bone resorption and cartilage degeneration via the aforementioned ways. Furthermore, FLS induced by IL-1β, TNF-α and IL-17 produce metalloproteinases, cathepsins, mast cell proteinases destroying extracellular matrix, and colony-stimulating factors (GM-CSF and M-CSF) supporting local expansion of macrophages. Activated FLS also secrete a high amount of RANKL, factor contributing to the differentiation of osteoclasts from macrophages. Moreover, FLS also produce other proteases (ADAMTs) and adhesive molecules such as cadherins and integrins, which play serious roles in signalling pathways during the tissue destruction (Bartok and Firestein, 2010; Ruscitti et al., 2015; Yoshitomi, 2019).

As regards tissue-degrading enzymes, metalloproteinases MMP-1, MMP-13 (collagenases) and MMP-3 (stromelysin) are the most crucial in RA pathophysiology. This huge group of zinc-dependent metalloendopeptidases is also detectable in CIA. *In vitro* studies confirm that the collaboration of TNF, IL-17A and IL-6 resulted in MMP-3 production by stimulated synovial fibroblasts (Slowikowski et al., 2020). IL-17 is also associated with increased secretion of MMP-13 (Shui at al., 2017), which seems to be the pivotal effector of cartilage degeneration. This enzyme targets collagen type II – a major component of the joint cartilage. MMP-9 is another enzyme destroying collagen tissue, and data from recent studies indicated its indispensable role in the migration of dendritic cells resulting in development of CIA and RA (He et al., 2018).

Conclusion

Cytokines play a fundamental role in each stage of CIA and RA development. Understanding how each member of this huge group affects the signalling pathways of the immune response is crucial for the development of new treatment agents. To evaluate the CIA severity, a large number of markers are used (cytokines, chemokines and antibodies). There is no general recommendation about the best cytokines for this assessment; each group of scientists use different markers related to their purpose. The most frequently cited cytokines are TNF-α, IL-1β, IL-6, IL-17 (IL-17A), which are followed by IL-2, IL-4, IL-8, IL-10, IL-12, IL-23, and GM-CSF and TGF-β. As was mentioned in the text above, the presence of each cytokine changes with the site of sample collection. We cannot say which of them is appropriate for a particular assessment because of the differences in the detected levels or actual presence of the cytokines in each cited paper. The final levels depend on the chosen devices, methods of detection and general conditions of the experiment.

Selective agents against cytokines are already applied in clinical practice – TNF, IL-1 and IL-6 inhibitors. Recently discovered GM-CSF inhibitors (Mavrilimumab, Otilimab) have not been registered yet by EMA. New IL-17 (IL-17A or IL-17F) inhibitors (Secukinumab, Ixekizumab) are registered for psoriatic arthritis and ankylosing spondylitis (EMA, 2023). Further discoveries are expected in research of the intracellular signal transduction process (as the latest drug class – inhibitors of JAK kinases) together with cytokines.

Conflicting of interests

The authors declare no conflict of interest.

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