UNIVERSITY OF GRONINGEN

Role of IL-23 in rheumatoid arthritis

Bachelor thesis
Pre-master Biomedical Sciences

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Januari 2016

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University of Groningen

January 2016

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Abstract

Rheumatoid Arthritis (RA) is a multifactorial, auto-immune disease. It is characterized by systemic inflammation, synovitis and movement-impaired joints. Genetic susceptibility and environmental, as well as a dysfunctional immune system play key roles in the progression of disease. IL-23, a cytokine belonging to the IL-12 cytokine family is essential for the differentiation an survivability of Th17 cells, a T lymphocyte involved in the production and secretion of the pro-inflammatory cytokine IL-17. IL-23 is known to be highly expressed in destructive synovial tissue in RA. The IL-23/IL-17 pathway could possibly underlie the pathogenesis of RA, which is unfortunately still not fully understood. The cytokine IL-23 is considered to be a promising therapeutical target in RA, which deserves more research.

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Introduction

Rheumatoid arthritis (RA) is a heterogeneous and currently incurable auto-immune disease affecting the synovial joints. Its prevalence is approximately 0.5-1% of adults and can, in severe cases, lead to joint deformities (1). Although its name was introduced in the 19th century (2), classification criteria were only developed recently (3). The complete pathophysiology of RA still needs to be defined, however, it is currently known that it is characterized by several mechanisms. In patients with RA, there is a progressive inflammation of the joints. In early stages of RA, the inflammation is local, however, due to the ability of synovial fibroblasts, the most abundant cell type in synovial tissues (4), to migrate *in vivo* to unaffected joints, systemic inflammation occurs (5). Destruction of cartilage involves these fibroblasts as well. Here, synovial fibroblasts also show abnormal behavior that is in line with join destruction, as they can invade normal human cartilage (1), which leads to an increase of friction between bone surfaces. In fact, the infiltration of these fibroblasts associates with the rate of joint destruction (6). Besides systemic inflammation and cartilage destruction, synovitis also plays a significant role in the pathophysiology and pathogenesis of RA. Because of hyperplasia, which involves an increased proliferation rate of cells within the synovium and an overproduction of synovial fluid (4),(7), the amount of damage to articular tissues increases. Ultimately, patients with RA suffer from pain from distended and movement-impaired joints.

Environmental and genetic factors are known to be the primary cause in developing RA, whereas 50-65% of risk is attributable to genetic factors (1),(8). Autoimmunity also plays a significant role. For genetic risk factors, heritability and the contribution of Human Leukocyte Antigen (HLA) type both play the major roles, whereas smoking is the highest environmental risk factor. Concerning autoimmunity, cytokines and self-antigens are key components in the immunopathology of RA.

Studies in the past decade are aiming to tackle RA, in which small biologic agents and small molecules, such as T/B-cells or cytokines, are being used as potential targets. Pro-inflammatory cytokines were recently implicated to play a fundamental role in the development of RA, as they promote inflammation and articular destruction.

IL-23, for example, a pro-inflammatory cytokine belonging to the IL-12 family, is secreted by activated dendritic cells and macrophages through an adaptive immune response. IL-23 is essential for the differentiation of T helper 17 (Th17) lymphocytes, and known to be upregulated in patients with RA, as shown in Figure 1 (9).

Th17 cells, a subset of differentiated naïve Cluster of Differentiation 4+ (CD4+) T cells (10), are crucial in mediating inflammatory and autoimmune diseases and are characterized by secretion of IL-17; another pro-inflammatory cytokine (11). Because of these findings, and since there is still little known about the exact cause of RA, this review focusses on IL-23, since it could play a key role in discovering the mechanisms behind complex diseases such as RA and other heterogeneous auto-immune diseases.

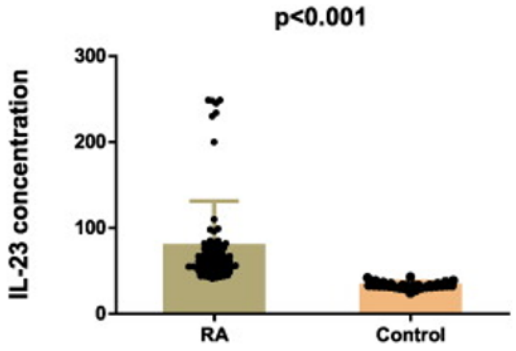

FIG. 1: Upregulation of IL-23 in patients with RA compared to healthy controls. (9)

There is currently a growing field of interest related to pathogenesis of disease. This has led to numerous studies examining the role of cytokines as novel therapeutic targets. However, there is still a lot of research to be done, since there is still little known about the possibilities . Therefore, in this literature study, it is hypothesized that the cytokine IL-23, involving effects of Th17 cells, underlie the pathogenesis of auto-immune diseases such as RA, and could potentially function as a novel therapeutic target in curing patients with RA.

Genetic and immunological contribution in the development of rheumatoid arthritis

As stated above, approximately 50-65% of risk factors are contributed by genetics in RA. Two specific types of HLA play significant roles in the genetic susceptibility to RA. In 1978, there was reported that up to 78% of Caucasian RA patients were HLA-DRw4 positive compared to 28% of controls (12). Secondly, HLA-DRB1 was suggested to play a very important role in the autoimmunity of RA, since the strongest association of this HLA type is with the development of anti- cyclic citrullinated protein antibodies (anti-CCP). Anti-CCPs are auto-antibodies, directed against citrullinated proteins that often occur in inflamed tissues. Citrullinated proteins or peptides are antigens which can cause the more violent immune reactions, and are known to be pathogenic in an animal model. Besides that, not only are citrullinated proteins important for initiation of RA, nearly 60-70% of RA patient test positive for presence of citrullinated proteins, indicating importance at the early stages of RA for diagnosis (13). Citrullinated proteins are present in rheumatoid synovium, indicating a correlation between HLA-DRB1 and RA.

Another genetic susceptibility is a polymorphism (gain of function mutation) in the protein tyrosine phosphatase nonreceptor 22 gene (PTPN22). In its downstream pathway, this gene can inhibit T cell activation and T cell receptor signaling, resulting in a defect in clearance of autoreactive T cells, contributing to the autoimmunity of RA.

Cytokines, T cells and B cells, and other major components of the adaptive immune response result in the characteristic inflammatory phenotype of RA. As mentioned before, when an auto-immune response initiates, antigen-presenting cells (APCs) such as DC's and macrophages bind to self-antigens. Upon binding, IL-23 is being secreted by activated APCs.

Transforming growth factor β (TGF-β), a secreted protein which contributes to several mechanisms in cells such as cell proliferation and tissue repair, is inactive in its latent form and unable to induce cellular activities. Latent TGF-β is present in cartilage and synovial fluid. However, latent TGF-β is activated through activation of synovial fibroblasts, and in context with inflammatory, TGF-β can initiate *de novo* differentiation of IL-17 producing T-cells (14). Subsequently, TGF-β and IL-23 promote naïve T cells to differentiate to Th17 cells and promote Th17 cell survival, which in turn amplifies the inflammatory and joint destruction by secreting IL-17. An overview of this cascade is given in Figure 2 (15).

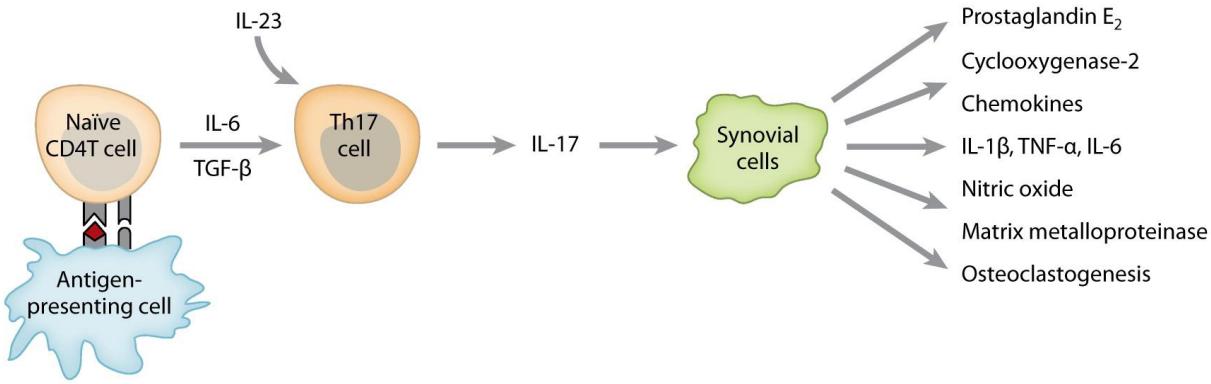

FIG. 2: Differentiation of naïve T-cells into IL-17 producing Th17 cells, where IL-17 can promote inflammation in different ways. Source: The Immunopathogenesis of Rheumatoid Arthritis. (15)

Natural regulatory T cells (Treg cells) are engaged in the maintenance of immunological self-tolerance and immune homeostasis. Their primary role is to suppress the immune system and prevent autoimmunity. It is known that defects in the function or depletion of levels of Treg cells are correlated to auto-immune diseases, since there is an impaired immunological response. In RA, Treg cells in peripheral blood of patients with RA function on an abnormal way. Compared to Treg cells in controls, Treg cells in patients with RA were unable to inhibit the production of pro-inflammatory cytokines, such as TNFα, by activated T-cells (16). Also, there is an imbalance between Th17/Treg cells in RA. In peripheral blood in patients with RA, this balance is broken, resulting in an increase of Th17 cells, a decrease of Treg cells and maintenance of inflammation (16)-(17).

Self-antigen clearance is hampered in RA. APC's are key components in these processes. On the contrary, in RA, activated macrophages release high mobility group box protein 1 (HMGB1), generally known as a highly conserved DNA binding protein involved in gene transcription (18), but can function as well as a pro-inflammatory cytokine mediator. Studies have shown that levels of HMGB1 were elevated in joints of rheumatic patients (19) and more recently that expression of HMGB1 could directly act on T cells to trigger an enhanced IL-17 production.

Toll-Like Receptors (TLR) function and expression is necessary for an optimal immune response. They are membrane receptors, and part of the innate immune system, particularly expressed on APC's. TLR's are activated by pathogen-associated molecular patterns (PAMPs), but are also able to recognize molecules associated with damage or cell destruction in joints, in other words, damage-associated molecular patterns (DAMPs). In RA, it is known that the expression of these membrane-spanning receptors are upregulated: TLR4 is highly expressed on blood monocytes in patients with RA (20). TLR's contribute to the pathogenesis by a positive feedback loop. On one hand, IL-17 is being released, and leads to an increase in the expression of several TLR's, including TLR4 (21). On the other hand, the function of TLR backfires in RA, as activated TLR on APC's, with interaction of HMGB1 as a TLR ligand, results in an upregulation of TLR4 and an increased production and release of cytokines in macrophages (22).

Both genetic susceptibility and a dysfunctional immune system are central in RA. IL-23 and other components, maintaining the survival and increase of differentiation of naïve CD4+ T cells into IL-17 producing T cells, respectively, form a complex system in which RA develops.

IL-23 in other diseases

As mentioned before, cytokines overall influence several heterogeneous diseases. RA and its primary reason to its inflammatory phenotype has already been described. In several studies, there was demonstrated that IL-23 not only plays a role in RA, but also in diseases such as Asthma and Inflammatory Bowel Disease, as will be described below. Now, what all these disease have in common is, of course, that inflammatory plays a significant role in its pathogenesis and development.

Allergic asthma is a chronic inflammatory disease which affects the airway epithelium. According to the World Health Organization, around 235 people suffer from asthma. Asthma is the most common disease among children and it is characterized by airway hyperresponsiveness, an exaggerated production of mucus and inflammation of the airway walls (23). All this leads to an limitation in airflow affecting the patients. Similar as in asthma, IL-23 promotes IL-17 secretion. IL-23 enhances maintenance of inflammation in asthma in an additional way. It also appears to mediate Th2 production, resulting in a disbalance between anti-inflammatory Th1 and pro-inflammatory Th2. This has been seen before in RA, which was regarding the disbalance in Treg/Th17(24). Activated DC's by exposure to allergens can either induce naïve T cells to differentiate to Th2 of Th17 cells, leading to different severities of allergic asthma. An overview of this mechanism is shown in Figure 3 (39).

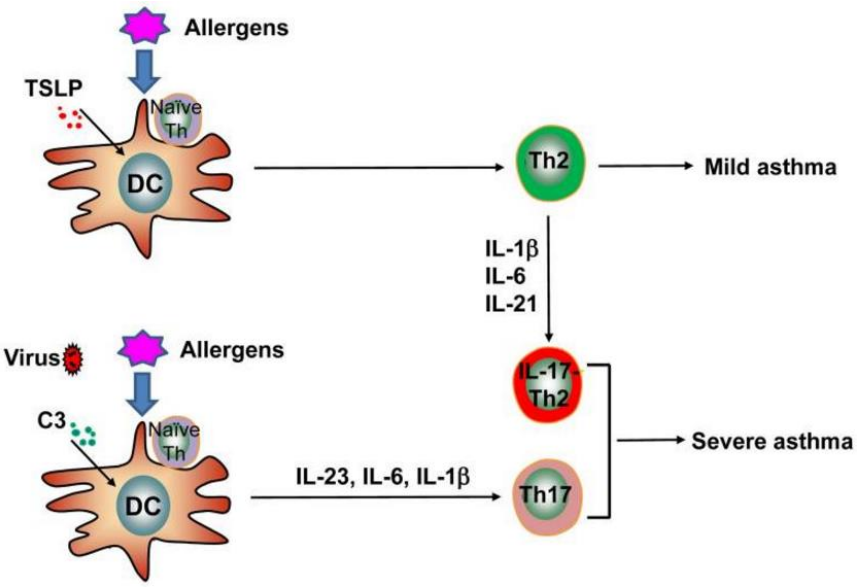

FIG 3: Schematic overview of allergic asthma development. Upon DC activation and secretion of differentiation of naïve T cells to Th2 or Th17 cells, results either in mild or severe asthma. (39)

Another chronic inflammatory disease in which the IL-23/IL-17 pathway is involved, is Inflammatory Bowel Disease (IBD). Similar to RA, it is an auto-immune disease affecting the intestines where both genetic and environmental factors contribute to the susceptibility. Interestingly, in a Danish cohort, polymorphisms in the IL23/IL-17 pathway as well as polymorphisms in TLR4 appeared to be correlated with a strong inflammatory response (25). The cost of NSAIDs vary in price, and are not always successful in bringing significant change after intake (26). Glucocorticosteroids, a class of corticosteroids, are synthetic hormones used in several physiological processes. In RA, they are used in treatment of swelling in joints and inflammation. The last group of anti-rheumatic drugs, DMARDS, are mostly being prescribed after positive diagnosis. Methotrexate (MTX), the most prescribed DMARD, prevents further permanent damage in untreated RA (27)-(28).

More recent therapies include medication in which small biological molecules are being used to suppress or inhibit physiological pathways or mechanisms involving in, for instance, the inflammation in RA. Technology led to the introduction of TNFα and IL-antagonists. TNFα inhibitors are monoclonal

antibodies suppressing the pro-inflammatory role of the cytokine TNFα. At this moment, the five most-used TNFα-antagonists are Infliximab, Adalimumab, Etanercept, Golimumab and Certolizumab Pegol (29). Only Etanercept is a genetically engineered protein, the rest are all monoclonal antibodies blocking the effects of TNFα.

However, these types of medications come hand in hand with, sometimes, severe immunogenicity through an immune response directing against therapeutic antibodies. The formation of these complexes between antigens and antibodies are called anti-drug antibodies (ADA), and can cause severe immunotoxic side-effects. All five biologic agents were associated with ADAs and its known that immunogenicity leads to a decrease in clinical response to these biologic agents (29). Immunogenicity differs, however, between the different types of TNFα antagonists.

Besides that, these therapeutics focused on remission, relieving pain and lessen joint swelling in patients with RA. It is therefore necessary that treatments are implied that intervene in -or manipulate the fundamental pathways causing RA.

Interleukin-inhibitors are the second group of small biologic molecules used in therapy. Because interleukins are involved in a very broad range of pathways, therapy started focusing on interleukins as potential targets in the pathogenesis of disease. This has been a hot topic in the past decade, and since then, numerous monoclonal antibodies were designed to specifically inhibit a cytokine receptor.

Immuno-intervention became a new field of research in auto-immune diseases. This kind of intervention was also applied in the pathogenesis of RA, where numerous antibodies were developed to manipulate naïve CD4+ T cell differentiation into IL-17 producing Th17 cells and counter Th17 cell survivability by influencing IL-23 function. An overview of the already designed antibodies involving in the inflammatory IL-17 pathway is shown in Figure 4 (25).

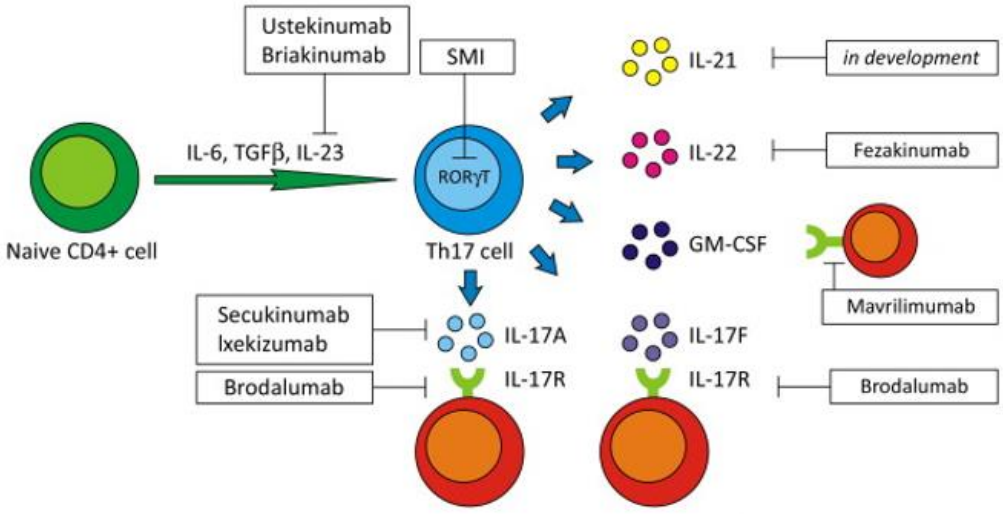

FIG. 4: Naïve CD4+ T cell differentiation into pro-inflammatory IL-17-producing Th17 cells. Therapeutic targets are shown by inhibiting cytokine involvement in this pathway (25)

As shown in the figure above, Ustekinumab, is able to inhibit IL-23 cytokine function. Ustekinumab is a monoclonal antibody and IL-23 receptor antagonist directed against a subunit (p40) of the IL-23 receptor. Inhibition of IL-23 function ultimately leads to a weakened inflammatory phenotype in RA. Additionally, research has brought to light that both IL-23 and IL-17 are present in serum, synovial fluid and synovial tissue in patients with RA, while both cytokines are absent in healthy joints (30).

Ustekinumab is currently being used in the treatment of psoriasis, a chronic auto-immune disease affecting the skin, in which inflammation also plays a key role. It has been proven to be a successful inhibitor of IL-23 function in psoriatic arthritis, a form of arthritis affecting patients with psoriasis (31). In Crohn's disease, a major category in IBD, Ustekinumab shown great promise as well, since it greatly increased disease remission (32).

However, in active RA, preliminary data of an annual meeting of the European League Against Rheumatism unfortunately shows that targeting the IL-23/IL-17 pathway by using Ustekinumab is failing in bringing significant benefits in treatment (33).

Although Ustekinumab resulted in no significant differences in RA, Briakinumab, another IL-23 inhibitor has not been tested yet in RA, still leaving potential of IL-23 inhibitors in RA (34).

Discussion

Rheumatoid arthritis is an enormous complex disease, with environmental and genetic factors affecting its initiation, development, and progression. Because of inflammation, leading to destruction of synovial tissue and cartilage in, what used to be, healthy joints, patients with RA are struggling. Additionally, there is no cure yet. Up to this point, there are no long-lasting therapeutics that intervene on the onset of RA, but only treatments that can cause remission, relieve pain and lessen joint swelling.

Genetic factors build up susceptibility, while cytokines and self-antigens promote auto-immunity in RA, eventually giving it its inflammatory phenotype. Local inflammation can become systemic inflammation due to invasive fibroblasts, resulting in swollen, movement-impaired joints.

Interleukin 23 plays a central role in RA, and its involvement in inflammatory has been reported my many studies. Based on clinical trials in chronic inflammatory diseases as Crohn's disease and Psoriasis, the IL-23/IL-17 pathway has established it to be a fundamental pathway in the development of disease. However, it is yet unclear how patients with RA are not affected by IL-23 antagonists, even if there was already confirmed that there was a upregulation of IL-23 in serum levels, and documented that both IL-23 and IL-17 are absent in healthy joints.

Even though this literature study mainly focused on the mechanisms in which T-cells are involved in the pathogenesis, B cells also play critical roles. B cells are the source of anti-citrullinated antibodies, giving the auto-immunity in RA a boost. Also, they are involved in T cell activation (35). Therefore, B-cell depletion therapy has been under investigation for many years and seems very promising (36). In the scope of this literature study, no pro-inflammatory cytokines were implied other than TNF-α, IL-23 and IL-17.

Future perspectives could be RNA interference (RNAi) or immunotherapy. Recent evidence in an asthmatic mouse model demonstrates that IL-23 silencing was established using IL-23 small interfering RNA-expression plasmid, successfully inhibiting IL-23 expression (37). As for immunotherapy, gene manipulation in DCs by siRNA resulted in downregulation of pro-inflammatory cytokines and upregulation of anti-inflammatory Treg cell activity in mice in which auto-immunity was induced (38). Both techniques could potentially give new insights in finding new therapeutic targets.

In conclusion, there is still a lot of research to be done to unravel the exact mechanisms in which IL-23 is involved in RA. Cytokines play major roles in different kinds of disease. Evidence suggests that IL-23 plays at the heart of RA, but, because of its complexity, and multifactorial traits, the question still remains to what extent IL-23, involving Th17 differentiation, orchestrates the pathogenesis in RA.

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Acknowledgements

I would like to gratefully and sincerely thank Prof. Dr. R. Gosens for providing me his guidance and positive feedback.