FoxP3 Expression in $CD4^{+}CD25^{high}$ T-cells in Rheumatoid Arthritis

Thesis

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Introduction

Autoimmune diseases affect about 5% of the world's population ***(Anderson and Isaacs, 2008).*** Rheumatoid arthritis **(RA)** is one of the most common human autoimmune diseases ***(Valencia et al., 2006).*** Its prevalence among adults is approximately 1% but varies across racial and ethnic groups ***(Scott and Kingsley, 2006).*** It affects individuals of middle age groups and occurs three times more frequently in women than in men ***(Ebringer and Rashid, 2006).***

Although breakdown of self-tolerance is a hallmark of RA, its etiology and pathogenesis are not fully understood ***(Han et al., 2008).*** It is characterized by chronic joint inflammation, the inflammation is located in the synovial tissue where the presence of proinflammatory cells and cytokines leads to damage to the cartilage and bone ***(Van Amelsfort et al., 2007).*** There is considerable evidence to support the involvement of T-lymphocytes in the pathogenesis of RA ***(Lawson et al., 2006).***

Regulatory T-cells **(T-regs)** are an indispensable cellular constituent of the normal immune system and they have crucial roles in establishing and maintaining immunologic self-tolerance and immune homeostasis ***(Sakaguchi et al., 2007).*** They are $CD4^+$ T-cells that constitutively express the receptor of α chain of interleukin **(IL)-**2 (CD25), only the $CD4^+$T-cells expressing the highest levels of CD25 ($CD25^{high}$) have *in vitro* suppressing activity. Changes in the number of T-reg cells or in their activity can be considered as possible causes for the

observed disturbances in immune regulation of patients with active RA ***(Chavele and Ehrenstein, 2011).***

The forkhead transcription factor **(FOXp3)**, specifically expressed in $CD4^+$ $CD25^{high}$ T-reg cells, is crucial for development of T-reg in the thymus and is needed to maintain the suppressive function of mature peripheral T-reg cells ***(Williams and Rudensky, 2007***).Therefore, FoxP3 is a specific molecular marker for T-reg cells in human peripheral blood. However, several reports describing $CD4^+CD25^{high}$ T-reg cells in peripheral blood of RA patients did not take FoxP3 into account. Therefore, it is necessary to detect FoxP3 expression by the $CD4^+CD25^{high}$ T-cells in RA patients ***(Han et al., 2008).***

Aim of the Work

To assess the frequency of FoxP3 in $CD4^{+}CD25^{high}$ T-reg cells in RA patients, as well as to correlate T-reg cells frequency in RA patients with disease activity.

Rheumatoid Arthritis

Rheumatoid arthritis **(RA)** is a chronic systemic inflammatory disease that may affect many tissues and organs, but principally attacks the joints in a systemic pattern producing an inflammatory synovitis that often progress to destruction of the articular cartilage and ankylosis of the joints ***(Majithia and Geraci, 2007).***

Turkcapar et al. (2006) classified RA as second most common rheumatic disease after osteoarthritis but it is the most destructive for synovial joint.

Joint damage in RA begins with the proliferation of synovial macrophages and fibroblasts after a triggering incident, possibly autoimmune or infectious. Over time, bone erosion and irreversible joint damage can occur, leading to permanent disability (*Fig. 1).* Although most readily recognized by its articular manifestations, multiple organ systems (like the lungs, pleura, pericardium, sclera and subcutaneous tissue) may be affected and may result in shortened life expectancy and worsening of the prognosis ***(Rindfleisch and Muller, 2005).***

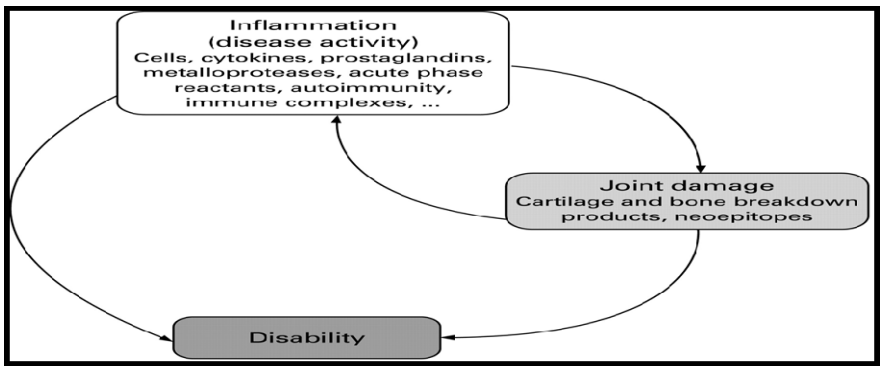

Figure (1): RA is a vicious cycle of events ***(Smolen et al., 2009).***

RA is associated with increased mortality rates compared with the general population, and the expected survival of RA patients is likely to decrease 3–10 years**.** Cardiovascular disease accounts for around (40%) of all deaths in RA, with cancer (17%), infection (14%), musculoskeletal disease (9%), respiratory disease (9%) and renal disease (6%) causing the majority of other deaths ***(Carmona et al., 2010).***

Although the earliest known appearance of RA was noted in skeletal remains of Indians from 4500 BC, no document evidence was found until much later. It wasn't until 1859 that the disease earned a proper name by Sir Alfred Garrod, the London physician, who coined the clinical term "rheumatoid arthritis". In 1941, RA became official. The American Rheumatic Association Officially recognized RA as distinct disorder ***(Canavese and Fogel, 2009).***

I) Epidemiology:

About 1% of the world's population is afflicted by RA, females 3 times more often than males. Onset is most frequent between the ages of 40 and 50, but people of any age can be affected ***(Majithia and Geraci, 2007).***

The disease beginning over 60 or 65 years is described as a late onset RA **(LORA),** and at middle age is defined as a younger onset RA **(YORA),** and both may differ significantly with respect to the mode of onset, the prevalence of associated systemic symptoms, the diagnostic criteria, the progression of diseases and the functional outcomes ***(Turkcapar et al., 2006).***

The incidence and the prevalence of RA vary across populations. The incidence in North America and Northern Europe ranges from 20 to 50 cases per 100,000 populations with prevalence of 0.5%. In Southern Europe, lower incidence of 9 to 24 cases per 100,000 populations and lower prevalence of 0.3% to 0.7% have been reported. The incidence of RA in developing countries is unknown but studies from these countries reported low prevalence between 0.1- 0.5%, while very low frequency of RA in some areas in rural Africa ***(Tobón et al., 2010).***

The prevalence of RA in rural Egypt is 0.29% similar to other oriental rural populations but lower than western populations ***(Abdel Tawab et al., 2004).***

II) Pathogenesis of RA:

RA is a heterogeneous disease or group of diseases. Despite many years of intensive investigations, the etiology of RA remains obscure. However, it is generally thought to be a combination of many factors including genetic, environmental, immunological, endocrinal and possibly viral factors ***(Gregersen and Olsson, 2009)***.

1) Predisposing factors:

a) Genetic factors:

Genetic factors have a substantial influence on determining the susceptibility to develop RA. Twin studies have demonstrated a fourfold higher concordance rate in monozygotic (15%) than in dizygotic (3.6%) twins. The risk in siblings of patients compared with that in a 'normal' population

has been estimated at between two- and 17-fold greater ***(Goronzy and Weyand, 2009).***

The gene most strongly associated with RA is the human leukocytic antigen DRB1 **(HLA-DRB1)** gene in the major histocompatibility complex **(MHC)**, it may contribute up to one-third of the genetic susceptibility to RA ***(Tobón et al., 2010).***

Among other genes, a polymorphism within the protein tyrosine phosphatase non-receptor 22 **(PTPN22)** gene has been associated with RA. The polymorphism is responsible for an amino acid exchange from an arginine to a tryptophan within the coding region of the gene. The resulting gain of function, with enhanced regulation of T cell receptor **(TCR)** signaling during thymic selection, permits autoantigen-specific T cells to escape clonal deletion, thereby predisposing to autoimmunity ***(Goronzy and Weyand , 2009).***

Substantial differences in genetic RA susceptibility factors have been found between European and Asian populations. HLA-DRB1 is the only known genetic factor that is associated with RA in all the populations studied. PTPN22 polymorphisms have been identified in European populations but are rarely found in Asian populations. On the contrary, polymorphisms of peptidylargininedeiminase 4 **(PAD4)** (which encodes an enzyme that converts the arginine residues of proteins into citrullines, which can then be recognized by anti-citrullinated protein antibodies **(ACPAs)** in the sera of RA patients) have been found more consistently in Asian than in European populations ***(Lee et al., 2009)***.

b) Environmental factors

RA is one of the complex immune-mediated diseases for which an understanding of the etiology is dependent on the definition of environmental triggers that, in a restricted genetic context, may initiate immune reactions having the potential to contribute to disease development ***(Klareskog et al., 2006).***

Environmental factors that affect RA may act many years before the disease becomes clinically apparent. The main environmental factors implicated in the development of RA include smoking, infections, dietary factors, pollutants, urbanization ***(Tobón et al., 2010).***

- ***Smoking:***

Among environmental factors, smoking has the strongest association with RA. Smoking increases susceptibility to RA and adversely affects the clinical course of the disease ***(Costenbader et al., 2008).***

Smoking is associated with rheumatoid factor **(RF)** and ACPAs production. It multiplies the adverse effect of the HLA-DRB1 shared-epitope alleles. Heavy cigarette smoking has been linked to a substantial increase in the susceptibility to RA. The risk of RA remains elevated up to 20 years after smoking discontinuation ***(Kobayashi et al., 2008).***

Smoking induces tissue damage and increases apoptosis through generation of free radicals, release of metalloproteinases, and the induction of Fas expression on lymphocytes. In addition, smoking induces inflammation as it causes elevation of fibrinogen

levels, induces leukocytosis, and elevates levels of C-reactive protein **(CRP),** intercellular adhesion molecule-1**(ICAM-1)** and E-selectin ***(Costenbader et al., 2008).***

- ***Infectious agents:***

Several microorganisms have been implicated in the development of RA based on higher titres of the relevant antibodies in patients with RA. One possibility is that these microorganisms trigger the development of RA in individuals who carry genetic susceptibility factors to the disease ***(Tobón et al., 2010).***

The role for microorganisms as initiating factors of RA remains controversial. Clearly, no single microorganism is responsible for the development of RA. Evidence supporting a role for parvovirus B19 includes the presence of viral DNA in the synovial fluid **(SF)**, synovial cells, and/or synovial tissue of RA patients. Sera from RA patients contain high titres of Epstein–Barr virus **(EBV)** antigens and of antibodies to latent and replicative EBV antigens. In addition, EBV RNA has been identified in B cells in synovial tissue from RA patients. Despite these findings, the evidence that microorganisms are involved in the development of RA remains inconclusive ***(Meron et al., 2010).***

Patients with active RA have a lower than normal cellular immune response to an EBV protein that is important for its replication, thereby allowing the virus to replicate, and results in a high EBV load ***(Westwood et al., 2006).***

Although therapy could also have an impact on this, the virus can continue to replicate even in the face of high antibody responses. This leads to a high antigen load, which, if accompanied by a significantly high antibody response, could easily result in a high level of immune complex **(IC)** formation analogous to an autoimmune response with the resulting stimulation of RF ***(Westwood et al., 2006).***

- ***Diet:***

Diet high in eicosapentaenoic acid (as fish oil) has a favourable effect on the outcome of RA, since such fatty acids compete with arachidonic acids, the latter of which are involved in inflammation. Vitamin D intake and tea consumption are associated with decreased risk, while coffee is associated with increased risk ***(Olendzki et al., 2011)***.

c) Sexual hormonal factors:

RA is predominant in females more than in males, perhaps due in part to the stimulatory effects of estrogen on the immune system. Estrogen inhibits T-suppressor cell function ***(Takagi et al., 2000).*** The use of the oral contraceptive pills **(OCPs)** and pregnancy are both associated with a decreased risk ***(Silman and Pearson, 2002).***

Pennell et al. (2012) demonstrated hypogonadal levels of testosterone in men with RA. In addition lower levels of the androgenic hormone dehydroepiandrosterone **(DHEA)** and higher concentrations of estradiol have also been found in men with RA, an effect that is not related to glucocorticoid therapy. Male sex also appears to affect disease phenotype.

Compared with female RA patients, male patients have significantly later disease onset, are more likely to be RF-positive, and have higher titres of anti-cyclic citrullinated peptide **(anti-CCP)** antibodies ***(Jawaheer et al., 2006).***

2) Immunopathogensis:

The characteristic pathophysiology in RA is the destruction of bone and cartilage due to persistent synovitis of unknown etiology. Large quantities of inflammatory cytokines such as tumor necrosis factor **(TNF)**, IL-1, and IL-6 are produced in inflamed synovial membranes, and these are deeply involved in the spread and persistence of the inflammation ***(Brennan and McInnes, 2008).***

RA pathogenesis involves complex humoral and cellular reactions including ICs formation, vascular reactions and infiltration of lymphocytes and monocytes into the synovium. These infiltrating cells and synoviocytes release pro-inflammatory mediators, which perpetuate inflammation and destruction through effects on other cell types in the synovium and peri-articular structures (*Fig. 2)* (***Dayer and Choy, 2010).***

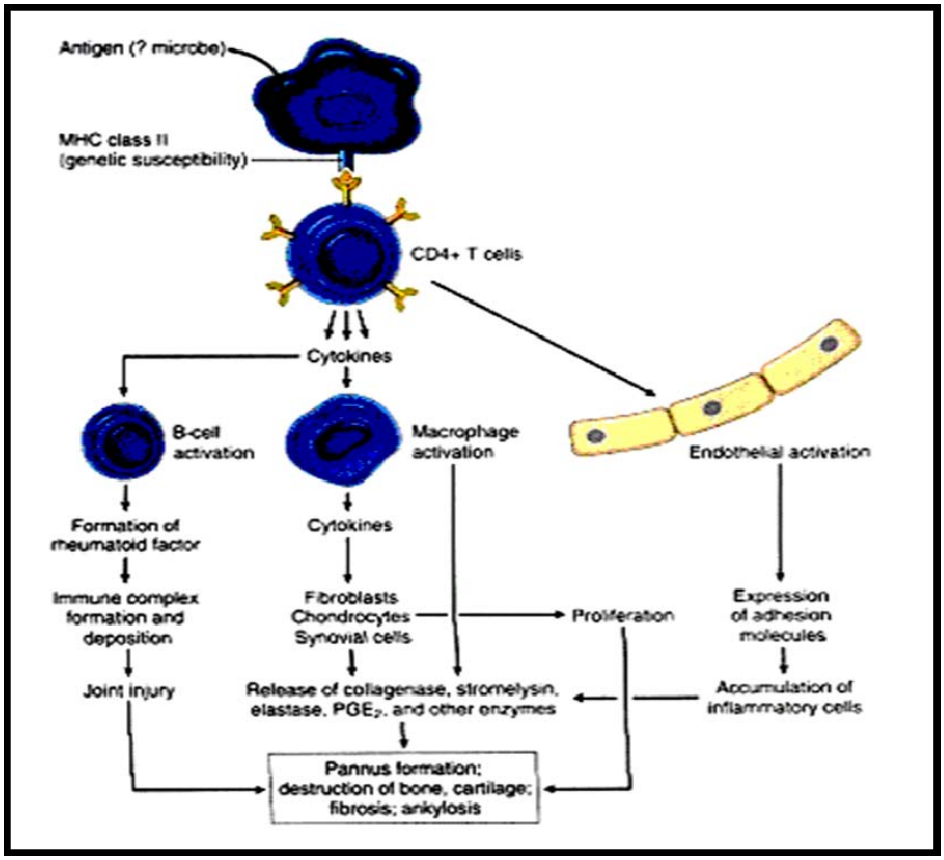

Figure (2): The immunopathogenesis of rheumatoid arthritis ***(Rosenberg, 2005).***

It is likely that, in genetically predisposed persons, an infective agent or another stimulus binds to Toll-like receptors **(TLRs)** on peripheral dendritic cells **(DCs)** and macrophages. This triggers a rapid response by the innate immune system involving cytokines and other inflammatory mediators, complement, natural killer cells **(NK)**, and neutrophils. DCs then migrate to lymph nodes, where they activate the adaptive immune system by presenting antigen to T cells ***(Scott and Kingsley, 2006).***

B cells also can function as antigen-presenting cells **(APCs)** leading to T cell activation. APCs communicate with T cells through the TCR–MHC interaction ***(Smith et al., 2011).***

T-cell activation requires two signals: signal 1 is generated by antigen (MHC –bound peptide on APC stimulates the TCR), whereas signal 2 is generated by CD28 costimulation (CD80 or CD86 on the APC interacts with CD28 on the T cell). These activated T cells proliferate and migrate into the joint, where they stimulate a multimolecular immune–inflammatory cascade. T cells produce Interferon gamma (**INF-γ)** and other proinflammatory cytokines, which stimulate macrophages, fibroblasts, chondrocytes, and osteoclasts. Activated macrophages and fibroblasts release TNF-α**,** IL-1, IL-6, IL-15, IL-18, and other proinflammatory cytokines that stimulate the production of additional inflammatory mediators (chemokines, prostaglandins **(PGs)**), proteases, and growth factors and activate neutrophils, B cells, and endothelial cells. Finally, joint damage, associated with the development of locally invasive pannus tissue, occurs through the actions of proteases, growth factors, and activated osteoclasts ***(Scott and Kingsley, 2006).***

Many RA patients have circulating antibodies to autoantigens other than IgG, including type II collagen, heat shock proteins, proteoglycans, cartilage link protein, and heavy chain binding proteins ***(Silverman and Carson, 2003).***

a) Role of T cells in RA:

T cells have been proposed to have a central role in the pathology of RA (*Fig. 3).* Although there are other

inflammatory cells present in the RA synovium, T cells are found in greater numbers than any other lymphocyte. The majority of T cells in the synovium are found forming clusters around perivascular areas and smaller numbers T cells can be found scattered in the RA synovium ***(Hidalgo, 2010)***.

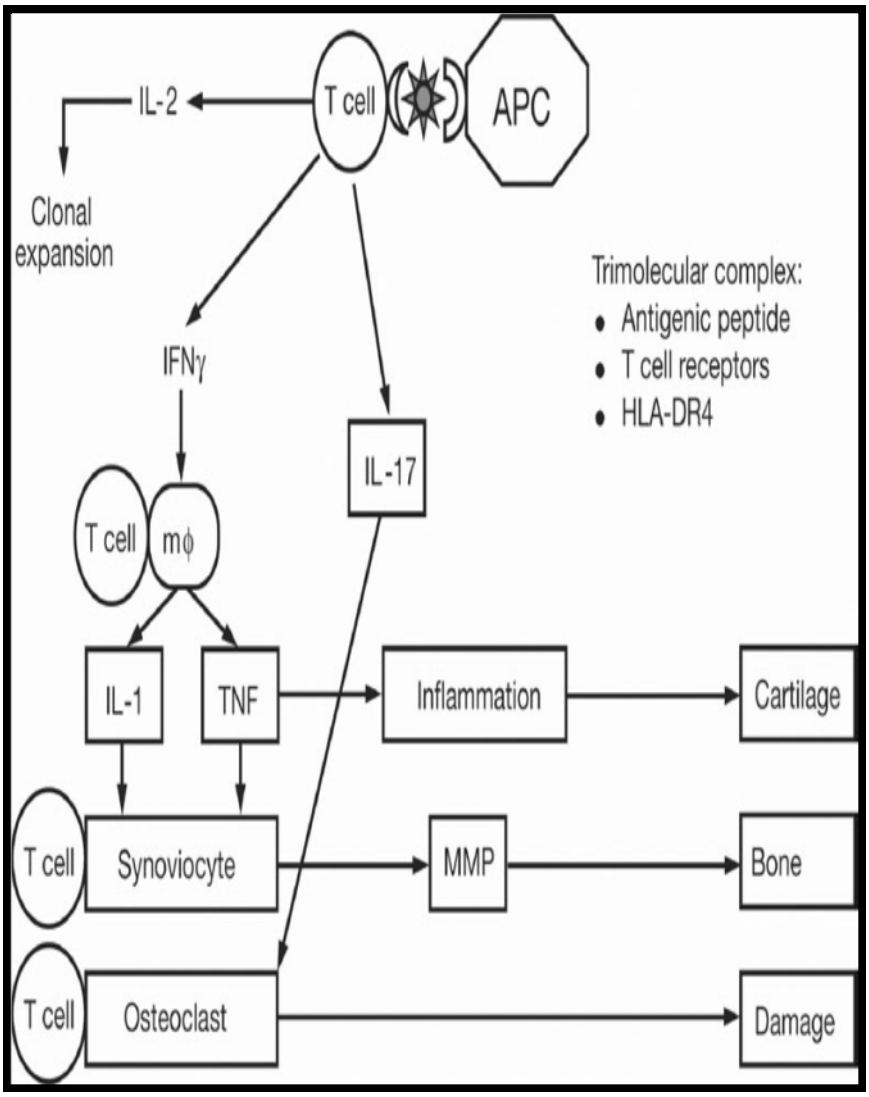

Figure (3): T cell-mediated pathways in rheumatoid arthritis ***(Panayi, 2005).***