INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint swelling, tenderness and destruction of synovial joints in addition to extra-articular manifestations leading to severe disability and premature mortality with incidence ranging from 5 to 50 per 100 000 adults in developed countries ***(Aletaha et al., 2010).***

RA imparts a massive burden on health services worldwide. Inspite of the big advances in the medical treatment of RA, uncontrolled active rheumatoid arthritis causes decreased quality of life and other comorbidities. Efforts to discover new target therapies have achieved considerable success ***(Liu et al., 2010).***

The dominant local cell populations in joints affected by rheumatoid arthritis are synovial and cartilage cells. Synovial cells can be divided into fibroblast-like (FLS) and macrophage-like synoviocytes. FLS show abnormal behavior in rheumatoid arthritis that correlates with joint destruction. They can spread between joints, suggesting how polyarthritis might develop ***(Scott et al., 2010).***

FLSs are believed to actively participate in the pathogenesis of RA. Their formation of new tissue, pannus, with aggressive invasion into the cartilage, directly contributes to the destruction of joint tissue. Moreover, FLSs are sources of

many factors involved in perpetuation of inflammation as basic fibroblast growth factor, angiogenic factor and vascular endothelial growth factors ***(Bartok and Firestein, 2010).***

The formation of new blood vessels "angiogenesis" is now recognized as a key event in the perpetuation of RA synovitis. Although many pro-angiogenic factors have been identified, vascular endothelial growth factor (VEGF) has a central role in the angiogenic process. There are expectations that oncologic therapies that block VEGF activity as bevacizumab (Avastin®) will also be applicable to RA to reduce synovial angiogenesis and pannus proliferation ***(Su and Chen, 2009)***.

Several guidelines for management of rheumatoid arthritis exist. The drug management of RA can be considered of two headings. The first is the relief of symptoms, with pain relief being the number one priority for patients. The second is modification of the disease process so that radiological progression, which is closely correlated with progressive functional impairment, can be retarded or stopped ***(Scott et al., 2010).***

Disease-modifying antirheumatic drugs (DMARDs) and biologic agents slow disease progression and can induce disease remission in some patients. Methotrexate (MTX) is the most commonly prescribed DMARD ***(Burch and Onysko, 2012)***.

The use of mesenchymal stem cells (MSCs; also known as multipotent mesenchymal stromal cells) has been proposed as a ‘magic bullet’ approach towards skeletal tissue regeneration. They are progenitors of multiple cell lineages, including bone, cartilage, muscle, fat and tendon. MSCs can be easily isolated and rapidly propagated in culture. These cells have also been shown to have immunosuppressive and healing capacities, and prevent fibrosis. ***(Djouad et al., 2009)***

Mostafa et al., (2009) showed the regenerative capacity of MSC transplantation in the regeneration of osteoarthritic cartilage in experimental animals.

AIM OF THE WORK

This is a pilot study designed to assess the role of human umbilical cord mesenchymal stem cell transplantation and the role of anti-vascular endothelial growth factor (bevacizumab (Avastin®)) versus conventional methotrexate therapy in treatment of adjuvant induced arthritis in rats.

PATHOGENESIS OF RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a common autoimmune disease that is associated with progressive disability, systemic complications, early death, and socioeconomic costs. The cause of RA is unknown. However, advances in understanding the pathogenesis of the disease have fostered the development of new therapeutics, with improved outcomes ***(Mclnnes and Schett, 2011).***

The hallmark swelling, bony erosions and synovial thickening reflect the underlying inflammatory and autoimmune processes. The interaction of environmental factors and genetic susceptibility leads to altered post-transcriptional regulation and self-protein citrullination early in the disease process ***(Gibofsky, 2012).***

Citrullination (enzymatic deimination of amino acid arginine, to give atypical amino acid citrulline) is a normal physiologic process in dying cells, and under normal circumstances, the cells do not come in contact with the immune system. When clearance is inadequate, however, peptidylarginine deiminase (PAD) enzymes and citrullinated proteins leak out of the dying cells and contact the immune system. The PAD enzymes citrullinate extracellular proteins

containing arginine, creating citrullinated antigens ***(van Venrooij et al., 2011).***

The altered, citrullinated native protein is considered foreign by the immune system, which results in the formation of autoreactive (autoreactive = directed against the self) antibodies, these autoantibodies attack the body's own tissue and induce an inflammatory reaction, the consequence of citrullination can thus be an autoimmune mechanism in rheumatoid arthritis ***(Gyorgy et al., 2006).***

The term anti-citrullinated protein antibodies, abbreviated as "ACPA", includes all those autoantibodies that are directed against naturally occurring citrullinated epitopes of proteins (for example, mutated citrullinated vimentin, MCV) or against synthetic citrullinated peptides. For example, synthetically generated "cyclic citrullinated peptides", CCP, are used as target antigens in various anti-CCP test systems ***(Vossenaar et al. 2004).***

The sensitivity of the anti-CCP antibody test for RA is about 70% and the specificity is about 95%. The higher the level of anti-CCP antibody, the higher the correlation with erosive joint disease, functional disability, and extra-articular disease.Interestingly, anti-CCP has been demonstrated in sera up to 10 years before the onset of articular symptoms in some patients who later develop RA ***(Nielen et al., 2004).***

GENETIC AND ENVIRONMENTAL FACTORS:

RA involves a complex interplay among genotype, environmental triggers, and chance. Twin studies implicate genetic factors in RA, with concordance rates of 15 to 30% among monozygotic twins and 5% among dizygotic twins ***(MacGregor et al., 2000).***

Genomewide analysis makes it clear that immune regulatory factors underlie the disease. The long-established association with the human leukocyte antigen (HLA)–DRB1 locus has been confirmed in patients who are positive for rheumatoid factor (RF) or anti-citrullinated peptide antigens (ACPA) as alleles that contain a common amino acid motif (QKRAA) in the HLA-DRB1 region, termed the shared epitope, confer particular susceptibility ***(Mclnnes and Schett, 2011).***

Many other identified risk alleles in ACPA positive RA consistently aggregate functionally with immune regulation implicating nuclear factor κB (NF-κB)-dependent signaling and T-cell stimulation, activation, and functional differentiation. Moreover, gene-gene interactions increase disease risk, as described between HLA-DRB1 and PTPN22 ***(Kallberg et al., 2007).***

Table (1): Candidate Genes with Single-Nucleotide Polymorphisms (SNPs) Linked to Rheumatoid Arthritis and Their Potential Function in Pathogenesis ***(Mclnnes and Schett, 2011)*:**

Candidate Gene and Pathway	SNP Locus	Function Relevant to Pathogenesis
T-cell activation		
HLA-DRB1†	6p21	HLA DRB1 allele (also known as the shared epitope) involved in MHC molecule–based antigen presentation and responsible for self-peptide selection and T-cell repertoire; first discovered and still by far the strongest genetic link to RA
PTPN22	1p13.2	Lymphocyte-specific non-receptor tyrosine phosphatase involved in regulation of activation threshold of lymphocytes; second genetic link described in RA
AFF3	2q11.2	Transcription factor for lymphoid development
CD28	2q33.2	Costimulatory molecule for T-cell activation
CD40	20q13.12	Costimulatory molecule that enhances interactions between T and B cells and increases autoantibody production
CTLA4	2q33.2	Costimulation suppressor that regulates interactions between T cells and antigen-presenting cells
IL2RA	10p15.1	High affinity receptor for interleukin-2 on lymphocyte subsets
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IL2	4q27	Cytokine that regulates activation of T cells, particularly regulatory T cells
IL-21	4q27	Cytokine that regulates differentiation of T cells, particularly Th17, and activation of B cells
PRKCQ	10p15.1	Member of the protein kinase C family that regulates T-cell and macrophage activation
STAT4	2q32.3	Transducer of cytokine signals that regulate proliferation, survival, and differentiation of lymphocytes
TAGAP	6q25.3	Rho-GTPase enzyme involved in T-cell activation
NF-κB pathway		
REL	2p16.1	Proto-oncogene member of the NF-κB family that regulates leukocyte activation and survival
TNFAIP3	6q23.3	Signaling protein and negative regulator of TNF-α–induced NF-κB activation
TRAF1	9q33.1	Regulator of TNF-α–receptor superfamily signaling (e.g., to NF-κB and JNK)
Other pathways		
BLK	8p23.1	B-lymphoid tyrosine kinase involved in B-cell receptor signaling and B-cell development
CCL21	9q13.3	Chemokine implicated in germinal-center formation
FCGR2A	1q23.2	Low-affinity IgG Fc receptor that regulates macrophage and neutrophil activation and immune-complex clearance
PADI4	1p36.2	Enzyme that converts arginine to citrulline, creating autoantigens in RA
PRDM1	6q21	Protein that acts as a repressor of β-interferon gene expression
TNFRSF14	1p36.32	TNF-α–receptor superfamily member with proinflammatory activity

GTPase denotes guanosine triphosphatase, **JNK** Jun N-terminal kinase, **MHC** major histocompatibility complex, **NF-κB** nuclear factor κB, **Th17** type 17 helper T cells, and **TNF-α** tumor necrosis factor α

Smoking and other forms of bronchial stress (e.g., exposure to silica) increase the risk of RA among persons with susceptibility HLA–DR4 alleles. Environmental stressors of pulmonary and other barrier tissues may promote posttranslational modifications, through peptidyl arginine deiminase, type IV (PADI4), that result in quantitative or qualitative alteration in citrullination of mucosal proteins ***(Klareskog et al., 2006).***

Infectious agents (e.g., Epstein–Barr virus, cytomegalovirus, proteus species, and Escherichia coli) and their products (e.g., heat-shock proteins) have long been linked with RA, and although unifying mechanisms remain elusive, some form of molecular mimicry is postulated. The formation of immune complexes during infection may trigger the induction of RF. Furthermore, rheumatoid arthritis appears to be associated with periodontal disease: Porphyromonas gingivalis expresses PADI4 and gastrointestinal microbiome ***(Kamphuis et al., 2005).***

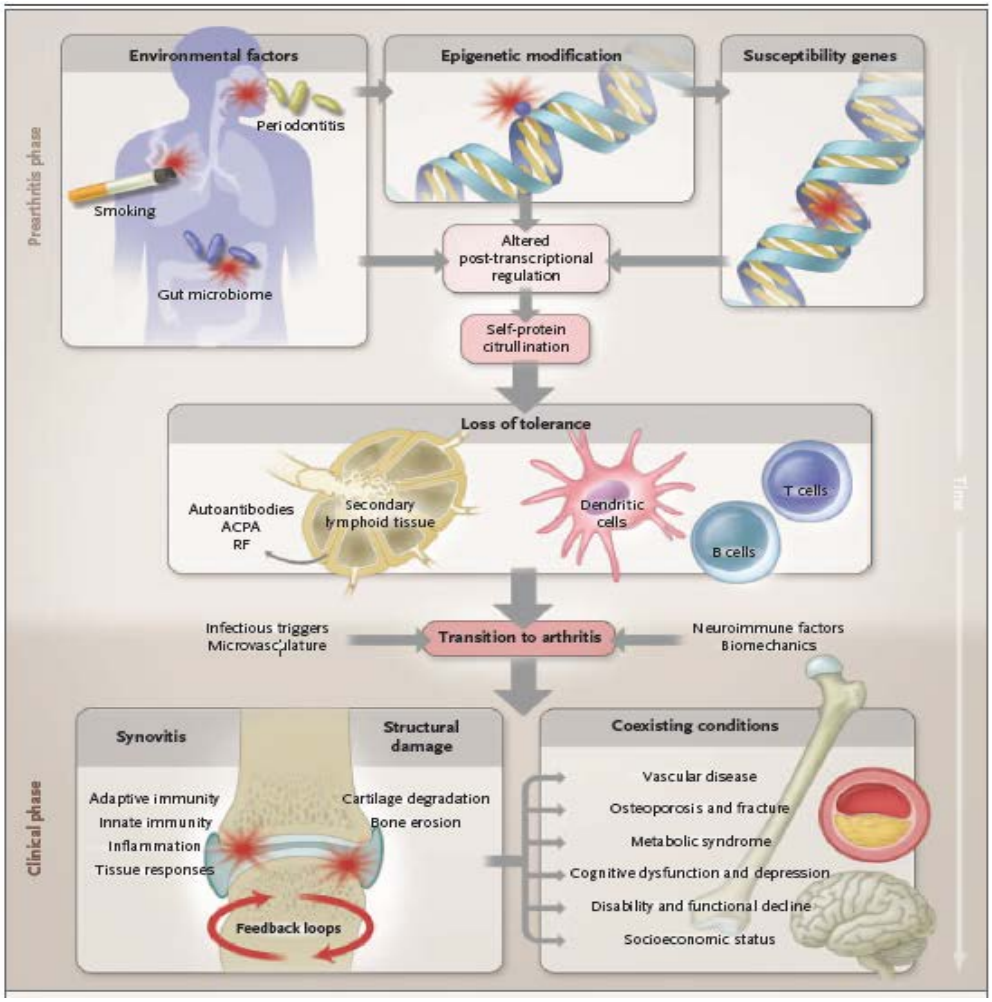

Figure (1): Multistep Progression to the Development of Rheumatoid Arthritis ***(Mclnnes and Schett, 2011)***

SYNOVIAL IMMUNOLOGIC PROCESSES AND INFLAMMATION:

Synovitis occurs when leukocytes infiltrate the synovial compartment which primarily reflects migration rather than local proliferation. Cell migration is enabled by endothelial activation in synovial microvessels, which increases the expression of adhesion molecules (including integrins,

selectins, and members of the immunoglobulin superfamily) and chemokines ***(Szekanecz et al., 2009).***

1) Activation of the Innate Immune System for RA:

A variety of innate effector cells, including macrophages, mast cells, and natural killer cells are found in the synovial membrane, whereas neutrophils reside mainly in synovial fluid. Macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and granulocyte–macrophage colony stimulating factor (GM-CSF) enhance maturation of these cells, their efflux from the bone marrow and trafficking to the synovium ***(Cornish et al., 2009).***

In particular, macrophages are central effectors of synovitis, they act through release of cytokines (e.g., TNF-α and interleukin-1, 6, 12, 15, 18, and 23), reactive oxygen intermediates, nitrogen intermediates, production of prostanoids and matrix-degrading enzymes, phagocytosis, and antigen presentation mainly through the predominantly M1 macrophage phenotype more than M2 macrophage phenotype ***(Haringman et al., 2005).***

Macrophages are activated by toll-like receptors (TLRs) (e.g., TLR 2/6, 3, 4, and 8) and nucleotide binding oligomerization domain (NOD)–like receptors (NLRs) that recognize a range of pathogen-associated molecular patterns and damage-associated molecular patterns that potentially

include bacterial, viral, and putative endogenous ligands ***(Seibl et al., 2003).***

Macrophage activation is also driven by cytokines, cognate interactions with T cells, immune complexes, lipoprotein particles and liver X–receptor agonists (e.g., oxysterols, oxidized low density lipoprotein [LDL], and serum amyloid A–rich high-density lipoprotein [HDL]), and the protease-rich microenvironment through protease activated receptor 2 ***(Liew and Mclnnes, 2002).***

Neutrophils contribute to synovitis by synthesizing prostaglandins, proteases, and reactive oxygen intermediates. Mast cells that produce high levels of vasoactive amines, cytokines, chemokines, and proteases, through ligation of TLR, suppression of tumorigenicity 2 (ST2), Fc receptor γ, and Fc receptor ε, also play a role. A fraction of ACPA belongs to the IgE class, which may elicit mast-cell activation through Fc receptor ε ***(Hueber et al., 2010).***

2) *Adaptive Immune Pathways for RA:*

The genetics of RA and the presence of autoantibodies clearly place adaptive immunity at the center of early pathogenesis. However, even though T cells are abundant in the synovial milieu, the functional role of T cells remains insufficiently understood ***(Panayiv, 2006).***

The synovium in rheumatoid arthritis contains abundant myeloid cells and plasmacytoid dendritic cells that express cytokines (interleukin-12, 15, 18, and 23), HLA class II molecules, and costimulatory molecules that are necessary for T-cell activation and antigen presentation ***(Lebre et al., 2008).***

Autoreactive T cells against citrullinated self-proteins have been identified. Synovial T-cell oligoclonality, germinal-center reactions, and B-cell hypermutation suggest ongoing local antigen-specific T-cell–mediated B-cell help ***(Cantaert et al., 2009).***

T cells require 2 signals for activation, where the first signal is antigen-specific and involves T-cell receptors and IL-2. The second signal, or costimulatory signal, involves interaction of CD80/86 on the antigen-presenting (dendritic cell) and CD28 on the T cell. Blockade of the costimulatory signal through competitive inhibition of CD80/86 prevents T-cell activation and the downstream events ***(Birbara, 2008).***

When T-cell activation does occur, T helper (Th) cells (eg, Th0, Th1, and Th17) are recruited. Although RA is conventionally considered to be a disease that is mediated by type 1 helper T cells, attention has increasingly focused on the role of type 17 helper T cells (Th17), a subset that produces interleukin-17A, 17F, 21, and 22 and tumor necrosis factor α (TNF-α). IL-17A works with TNF-α to promote the activation of fibroblasts and chondrocytes ***(Miossec et al., 2009).***

Th17 cells also trigger humoral adaptive immunity mediated by synovial B-cells. B-cells are triggered by factors including a proliferation-inducing ligand, B-lymphocyte stimulator, and CC and CXC chemokines.1 B cells secrete autoantibodies, present antigens to T cells, and stimulate synovial fibroblasts through the secretion of cytokines (eg, lymphotoxin-β [Ltβ] and TNF) ***(Isaacs, 2010).***

Macrophage-derived and dendritic-cell–derived transforming growth factor β and interleukin-1β, 6, 21, and 23 provide a milieu that supports Th17 differentiation and suppresses differentiation of regulatory T cells, thus shifting T-cell homeostasis toward inflammation. Regulatory (forkhead box P3 [Foxp3+]) T cells that are detected in tissues from patients with rheumatoid arthritis appear to have limited functional capability ***(Genovese et al., 2010).***

An additional pathogenic pathway comprises antigen-nonspecific, T-cell contact-mediated activation of macrophages and fibroblasts, operating through interactions between CD40 and CD40 ligand, CD200 and CD200 ligand, and intracellular adhesion molecule 1 and leukocyte-function-associated antigen 1 ***(Mclnnes et al., 2000).***

Humoral adaptive immunity is integral to rheumatoid arthritis. Synovial B cells are mainly localized in T-cell-B-cell aggregates. Indeed, some tissues have ectopic lymphoid follicles that are supported by the expression of factors that

include a proliferation-inducing ligand (APRIL), B-lymphocyte stimulator (BLyS), and CC and CXC chemokines (e.g., CXC chemokine ligand 14 and CC chemokine ligand 21). Plasmablasts and plasma cells are more widely distributed in the synovium and also in juxta-articular bone marrow ***(Seyler et al., 2005).***

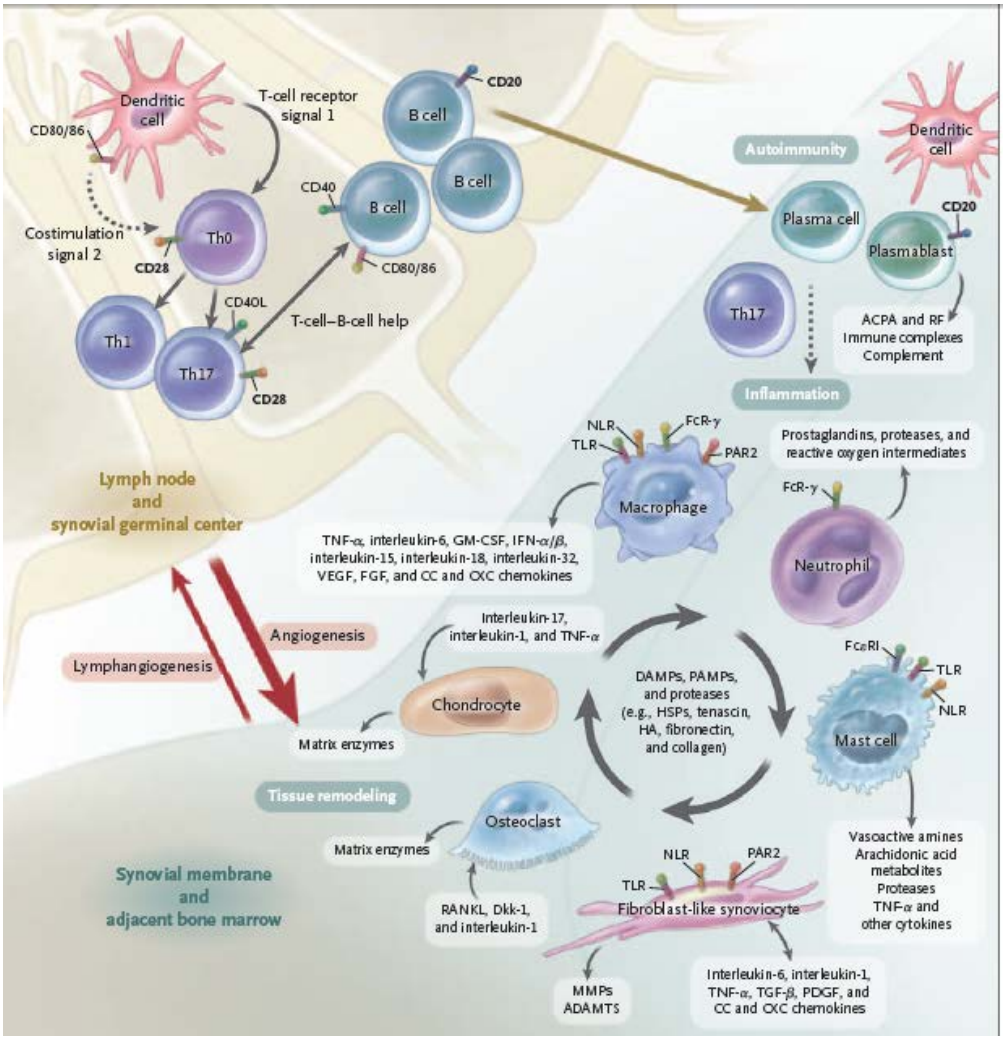

Figure (2): Adaptive and Innate Immune Processes within the Joint in Rheumatoid Arthritis ***(Gibofsky, 2012)***