# THE ROLE OF ADRENOMEDULLIN IN RHEUMATOID ARTHRITIS

Thesis

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By

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# INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disease primarily characterized by bilateral symmetrical polyarticular arthritis, which is often erosive. It is a common autoimmune disease (about١٪ of the world population), affects three times as many women as men, and usually appears in middle age ***(Hannah and Peter, ٢٠٠٧).***

This disease is characterized by chronic synovial inflammation and synovial cell proliferation producing the pannus, which can be compared with a local tumor responsible for bone and cartilage destruction. Pannus development is related to mononuclear cell infiltration, neoangiogenesis, and abnormal proliferation of type-B synoviocytes or fibroblast-like synoviocytes (FLS**) (*Benjamin et al., ٢٠٠٨).***

The pathogenesis of the rheumatoid pannus, although incompletely understood, has been partly explained by new knowledge on FLS biology ***(Pap et al., ٢٠٠٠a).***

Two general mechanisms contribute to synovial hyperplasia: increased FLS proliferation and decreased synoviocyte apoptosis. Increased expression of vascular endothelial growth factor (VEGF) indicates increased blood vessel density, lymphocytic infiltrate and subsequent increase in angiogenesis and pannus formation ***(Badr et al., ٢٠٠٥)***.

Type-A synoviocytes rarely undergo apoptosis ***(Firestein et al., ١٩٩٥)*** and other cells, including FLS ***(Kawakami et al., ١٩٩٩)*** and infiltrating T lymphocytes ***(Makino et al., ٢٠٠٣)***, express high levels of antiapoptotic molecules (such as bcl-٢) and resist Fas-induced apoptosis, suggesting a strong anti-apoptotic effect in the RA joint.

Extensive investigations into a deficiency in the Fas-induced apoptosis signaling pathway suggest possible modulation by other molecules such as sentrin ***(Pap et al., ٢٠٠٠b).*** Expression of a mutant p٥٣ tumor suppressor gene is another important mechanism of resistance to apoptosis ***(Badr et al., ٢٠٠٥)***. Other regulatory mechanisms may involve anti-apoptotic molecules such as growth factors (٥, ١٠, ١١), cytokines (e.g., interleukin (IL)-١٠ and IL-١٣), and adrenomedullin (AM) ***(Benjamin et al., ٢٠٠٨).***

Adrenomedullin (AM) is a ٥٢-amino acid peptide first identified in ١٩٩٣ in human pheochromocytoma. This peptide contains two structures important for its activity: a loop of six amino acids formed by a disulfide bond between residues ١٦ and ٢١ and an amide bond on the C-terminal tyrosine residue ***(Beltowski and Jamroz, ٢٠٠٤).***

The (٢٢-٥٢) AM truncated peptide does not have the six-amino acid ring and can act as an antagonist of the AM receptor, depending on the cell type and species. The mature AM is

synthesized as glycine extended AM followed by C-terminal amidation to assume a biologically active form in tissues, recently pro-inflammatory cytokines, such as tumor necrosis factors α (TNFα) and IL-١, were found to stimulate production and secretion of AM from vascular endothelial cells and vascular smooth muscle cells in vitro, suggesting that AM interacts with the immune system ***(Sugo et al., ١٩٩٥).*** However, AM reduces the production of TNFα from macrophages stimulated with lipopolysaccharide. In addition, AM shows an anti-inflammatory effect that reduces the production of the IL٨ family by macrophages ***(Kamoi et al., ١٩٩٥).***

In FLSs, AM binds to a heterodimeric plasma membrane receptor composed of the seven-transmembrane domain protein calcitonin receptor-like receptor (CALCRL) coupled to one of two receptor activity-modifying proteins (RAMP-٢ or -٣). CALCRL signal transduction is mediated through G protein-coupled adenylatecyclase (AC) and protein kinase A (PKA) pathways ***(Naot and Cornish, ٢٠٠٨)***.

Adrenomedullin (AM) is secreted by monocytes and macrophages ***(Kubo et al., ١٩٩٨)***, as well as by skin fibroblasts ***(Isumi et al., ١٩٩٨ b).*** It is expressed not only in the cardiovascular system, brain, kidneys, and lungs, but also in bone and joint structures including cartilage ***(Chosa et al., ٢٠٠٣)*** and synovium ***(Matsushita et al., ٢٠٠٤).***

Adrenomedullin (AM) stimulates angiogenesis and growth of connective tissue-derived cells and organs ***(Belloni et al., ٢٠٠١)*** and exerts antiapoptotic effects on various cell types ***(Benjamine et al., ٢٠٠٨).***

Interestingly, AM has several other effects, depending on the cell type: it inhibits smooth muscle cell migration ***(Horio et al., ١٩٩٥)***, increases cell proliferation ***(Zudaire et al., ٢٠٠٣),*** and stimulates human umbilical vein endothelial cell (HUVEC) proliferation and migration, as well as angiogenesis in vitro ***(Kim et al., ٢٠٠٣)*** and in vivo ***(Nikitenko et al., ٢٠٠٦)***.

Adrenomedullin (AM) also induces the expression of adhesion molecules like intercellular adhesion molecule (ICAM) and endothelial selectin (E-selectin) at the surface of HUVECs ***(Hagi-Pavli et al., ٢٠٠٤)*** and human oral keratinocytes ***(Hagi-Pavli et al., ٢٠٠٥).***

Several reports support a role for AM in the pathogenesis of RA. Plasma AM levels are higher in RA than in other inflammatory diseases (systemic lupus and scleroderma), osteoarthritis (OA), and in normal individuals ***(Yudoh et al., ١٩٩٩).*** Furthermore, AM levels in synovial fluid are higher in RA than in OA ***(Chosa et al., ٢٠٠٣).***

# AIM OF THE WORK

The aim of this work was to study the possible role of AM in the pathogenesis of RA by measuring its plasma level and relating it to disease activity and severity.

# RHEUMATOID ARTHRITIS

## Definition:

Rheumatoid arthritis (RA) is a chronic, systemic, progressive autoimmune disease, that 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 ***(Hekmat et al., ٢٠١١)***.

It is the most common type of inflammatory arthritis, with considerable impact on patient's life, on their families and on society as whole ***(Majithia and Geraci, ٢٠٠٧).***Extra-articular complications also often occur, thereby eliciting a worsening of the prognosis ***(Okuda, ٢٠٠٨).***

## Synovial pathology:

The normal synovium consists of an intimal lining layer that is usually discontinuous, one to two cell layers thick, and lacks an underlying basal membrane. The sublining below the intima contains blood vessels, lymphatics, nerves, and adipocytes distributed within a less cellular fibrous matrix. The intimal lining layer comprises roughly equal proportions of two different cell types, macrophage like synoviocytes or types A synoviocytes, and fibroblast like synoviocytes (FLS) or type B synoviocytes ***(Waldenburger and Firestein, ٢٠٠٨)*** **(Fig. ١).**

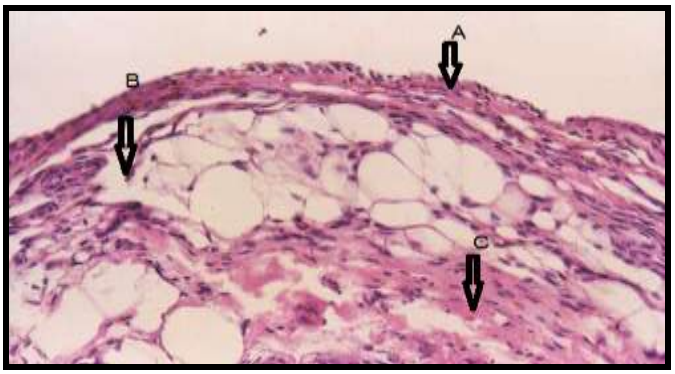


**Fig. (١):** Normal synovium showing A- flat synovial lining, B- mild vascularity, C- free lymphocytes ***(Dessouki et al., ٢٠٠٦).***

Rheumatoid arthritis (RA) causes a broad spectrum of morphologic changes being severely manifested in the joints. Initially the synovium becomes grossly edematous, thickened, and hyperplastic, transforming its smooth contour to one covered by delicate and bulbous fronds ***(Rosenberg, ٢٠٠٥).***

Neoangiogenesis is a dominant and consistent pathological feature that occurs early in the disease, present in all biopsies from patients with different stages of RA, and correlates with disease activity irrespective of disease duration ***(Buckly et al., ٢٠٠٥).***

**The characteristic histopathological features include** :

- Infiltration of synovial stroma by dense perivascular inflammatory cells, consisting of B cells and CD٤+ helper T cells (often forming lymphoid follicles), plasma cells, and macrophages.
- Increased vascularity owing to vasodilatation and angiogenesis, with superficial hemosiderin deposits.

- Aggregation of organizing fibrin covering portions of the synovium and floating in the joint space as rice bodies.
- Accumulation of neutrophils in the synovial fluid and along the surface of synovium but usually not deep in the synovial stroma.
- Osteoclastic activity in underlying bone, allowing the synovium to penetrate into the bone forming juxta-articular erosions, subchondral cysts, and osteoporosis.
- Pannus formation (which is a mass of synovium and synovial stroma consisting of inflammatory cells, granulation tissue, and fibroblasts), and growing over the articular cartilage causing its erosion**.** By time, after the cartilage has been destroyed, the pannus bridges the opposing bones, forming a fibrous ankylosis, which eventually ossifies leading to bony ankylosis ***(Rosenberg, ٢٠٠٥)*** **(Fig.٢ and ٣).**

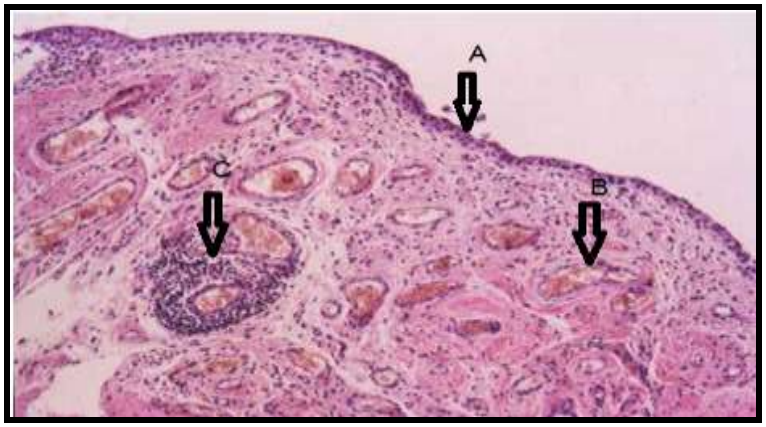


**Fig. (٢):** Synovium in RA showing A- synovial hyperplasia, B- increased mean vascular density, C- perivascular lymphocytic infiltration ***(Dessouki et al., ٢٠٠٦)***.

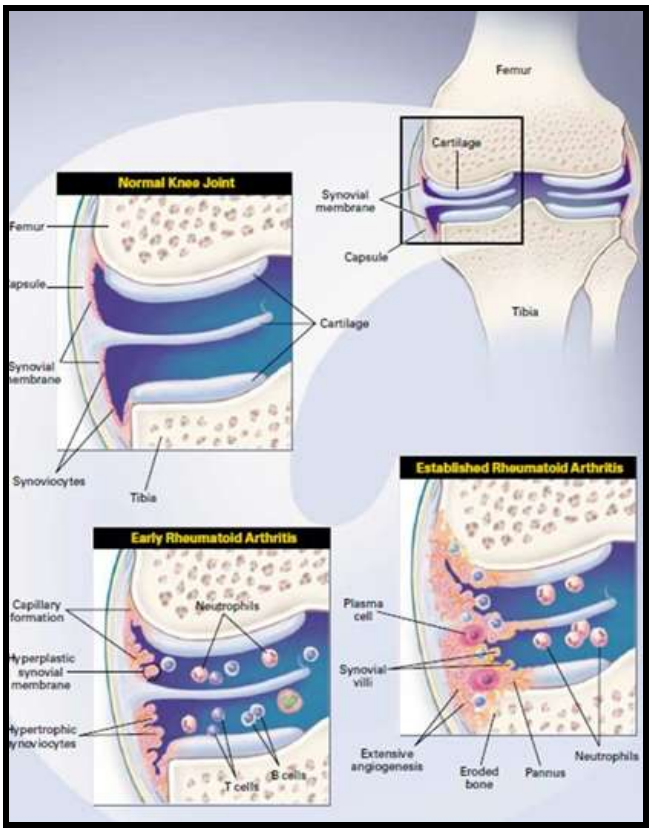


**Fig. (٣):** Schematic presentation of normal knee joint (a); joint in early RA (b); joint in established RA (c) ***(Choy and Panayi, ٢٠٠١)***.

## Immunopathogenesis of rheumatoid arthritis:

Pathogenesis of RA involves complex humoral and cellular reactions including immune complex (IC) formation, vascular reactions and infiltration of lymphocytes and monocytes into the synovium. These infiltrating cells and synoviocytes release pro-inflammatory mediators and multiple chemokines which perpetuate inflammation and destruction through effects on other cell types in the synovium and peri-articular structures (***Dayer and Choy, ٢٠١٠).***

**Role of T lymphocyte:**

The understanding of how T lymphocyte participate in the pathogenesis of RA is evolving rapidly with fundamental new insights into basic T-cell biology and the orchestration and regulation of immune responses. T cells are central component of organ-focused immune-mediated pathology, capable of interactions not only with classical cells of the immune system but with tissue-specific cell populations that contribute to inflammation and tissue destruction ***(Lundy et al., ٢٠٠٧).***

Engagement of T cells by self antigens within the thymus induces deletion of potentially harmful T cells by apoptosis. Defects in apoptosis lead to the persistence of autoreactive T cells recognizing self antigens which can induce autoimmunity ***(Marleau and Sarvetnick, ٢٠٠٥).***

***Bisgin et al.*** in ٢٠١٠, reported that during the development of RA, $CD^{4+}$ T cells initiate and regulate several cell-mediated immune processes that cause synovial inflammation and joint destruction in response to activation by antigen presentation. $CD^{4+}$ T cells obtained from RA patients exhibited higher levels of expression of TNF related apoptosis inducing ligand (TRAIL) and its death/decoy receptors compared to cells from control individuals.

T cells are abundant in active RA, comprising ٢٠٪-٥٠٪ of cells in the inflamed synovium. Activated T cells contribute

to proliferation and differentiation of B-cells and to the production of antibodies, macrophage activation and cytokine production, endothelial activation and expression of adhesion molecules, and regulation of osteoclast activation and thus joint destruction ***(Taylor,*** ٢٠٠٨***).***

Successful T-cell activation requires multiple signals**.** One signal is provided by presentation of an antigen bound to cell surface major histocompitability complex (MHC) molecules on antigen-presenting cells to a specific T-cell receptor (TCR). Co-stimulation of naïve T cells through ligation of CD٢٨ by B٧-١ (CD٨٠) or B٧-٢(CD٨٦) is perhaps the most important secondary signal to drive T-cell proliferation and differentiation. In the absence of further co-stimulatory signals, T cells become unresponsive and may be eliminated through apoptosis. Once activated, the T cell upregulates expression of cytotoxic T lymphocyte antigen-٤ (CTLA-٤), an inhibitory receptor that has a higher affinity for CD٨٠ and CD٨٦, in order to modulate activation ***(Taylor,*** ٢٠٠٨***)*** **(Fig. ٤ and ٥).**

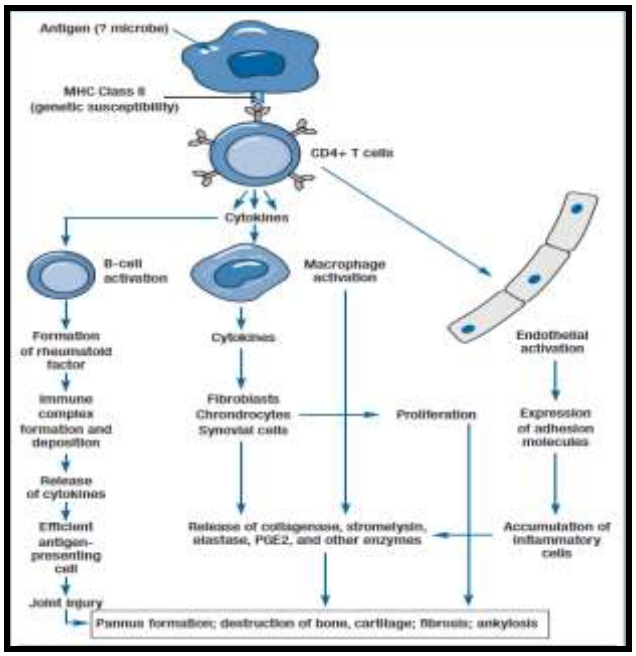


**Fig. (٤):** The immunopathogenesis of rheumatoid arthritis ***(Rosenberg, ٢٠٠٥).***

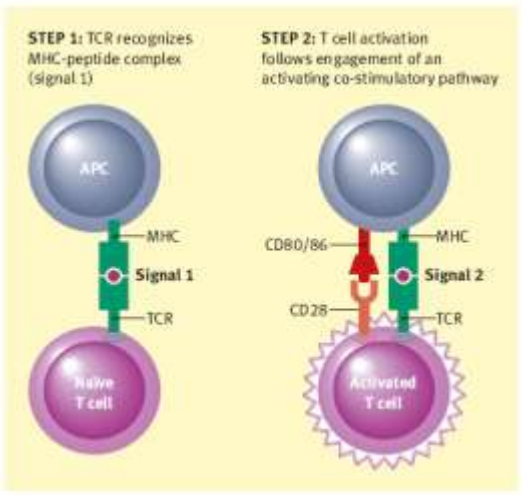


**Fig. (٥):** The T-cell activation ; TCR binds to and recognizes components of both the MHC and peptide to elicit signal one. Activation follows engagement of a costimulatory pathway. CD٨٠/٨٦:CD٢٨ is the best characterized co-stimulatory pathway. CD٢٨ is constitutively expressed on T cells and binds toCD٨٠/٨٦. CD٨٠/٨٦:CD٢٨ facilitates T-cell activation, proliferation, survival and cytokine production.TCR: Tcell receptor, MHC: major histocompitability complex ***(Taylor,*** ٢٠٠٨***).***

**Role of B-lymphocyte:**

B-lymphocytes play several critical roles in the pathogenesis of RA. They are the source of the rheumatoid factors (RF) and anti-citrullinated protein antibodies (ACPAs), which contribute to IC formation and complement (C) activation in the joints. B cells are also very efficient antigen-presenting cells and can contribute to T cell activation through expression of co-stimulatory molecules. B cells both respond to and produce the chemokines and cytokines that promote leukocyte infiltration into the joints, formation of ectopic lymphoid structures, angiogenesis, and synovial hyperplasia ***(Silverman and Carson, ٢٠٠٣).***

The presence of C in serum and synovial fluid of RA patients might contribute to this articular damage through different mechanisms, such as C activation. Therefore, identification of the antigens from these IC is important to gain more insight into the pathogenesis of RA. Since RA patients have antibodies against citrullinated proteins (ACPA) in their serum and synovial fluid (SF) and since elevated levels of citrullinated proteins are detected in the joints of RA patients, citrullinated antigens are possibly present in IC from RA patients ***(Van Steendam et al., ٢٠١٠).***

The formation of IC as such is not specifically related to autoimmune pathologies as it is a natural process, completing an immune response in the body. The antigen-antibody complexes are