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Clinical Laboratory

RHEUMATOID ARTHRITIS: ETIOPATHOGENESIS AND MOLECULAR BASIS

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ABSTRACT Rheumatoid arthritis (RA) is the most common form of inflammatory arthropathy sustained by autoimmune responses. This review has the objective of updating the knowledge about RA especially its molecular pathogenesis. We examine here the current knowledge of tryptophan, arginine, homoarginine and histidine metabolism and the main immunoregulatory pathways in amino acid catabolism in both RA patients and experimental models of arthritis. Of the characteristic autoantibodies of RA, those that appear earlier, are those that recognize cyclic citrullinated peptides. (CCP) and/or citrullinated fibrinogen. Therefore our analysis would indicate that amino acids metabolism represents a fruitful area of research for new drug targets for a more effective and safe therapy of RA.

KEYWORDS : Rheumatoid arthritis, tryptophan, homoarginine, histidine, arginine

INTRODUCTION

Rheumatoid arthritis is the most frequent of human systemic autoimmune diseases. It is characterized by chronic inflammation of the synovial joints with synovial proliferation and infiltration by blood-derived cells, particularly memory T cells, macrophages and plasma cells. Angiogenesis is a feature of the early stages of the disease. Synovial tissue becomes hyperplastic and invasive locally at the interface of cartilage and bone, with progressive destruction of these tissues. This invasive tissue is called "pannus"; there is also erosion of the bone, joint deformity and functional disability of the patient. The destruction of bone and cartilage is mediated by degradation enzymes, mainly metalloproteinases. Different cells of the innate and adaptive immune system show alterations in the expression of various genes that code for proteins (1). In addition RA can result in inflammation in the lungs, pericardium, pleura and sclera. A fundamental abnormality in RA is the inappropriate growth of immune cells and stromal cells, imposing high metabolic demands to generate energy and biosynthetic precursors. (2)

The presence of a wide variety of circulating autoantibodies has been described, including rheumatoid factor (RF) and antibodies to nuclear or structural cellular components. In addition antibodies against Epstein Barr virus (EBV) proteins have been frequently observed. Everything indicates that RA is an autoimmune process dependent on CD4 lymphocytes which induce chronic synovial inflammation. Several autoantibodies have been found to be present in the serum of RA patients. The autoantibodies characteristic of RA, and that appear earlier are those that recognize cyclic citrullinated peptides (CCP) and/or citrullinated fibrinogen.(3) Citrullination is a post-translational modification and has the potential to alter the structure, antigenicity and function of proteins such as fibrinogen, vimentin, type ll collagen. Citrulline is a modification of arginine and corresponds to an amino acid that is not incorporated into the polypeptide chain during the synthesis process, but is generated from a post-translational modification of arginine by the enzyme peptidylarginine deiminase that converts peptidylarginine into peptidylcitrulline. These auto-antibodies may be derived from the destruction of synovial cells containing filaggrin or more probable containing cross reactive molecules. (4)

We make a brief overview of the current knowledge of tryptophan, histidine and arginine metabolism and the main immunoregulatory pathways in amino acid catabolism, in both RA patients and experimental models of arthritis. The function of the L-arginine metabolism in RA is less clear. Amino acid metabolism represents a fruitful area of research for new drug targets for a more effective and safe therapy of RA.

ETIOLOGY

RA is one of the most frequent inflammatory joint diseases; has a worldwide distribution and affects all racial and ethnic groups. The prevalence in the United States of America is 0.3 to 1.5% of the population and despite its prevalence it has not been possible to clarify for years many aspects of its etiopathogenesis . Recently new aspects of the pathogenesis of RA have been discovered. Women are affected 2 to 3 times more than men. The disease can occur at any age and its incidence increases with age, mainly between 40 to 60 years of age; although it can occur at any age. Before the age of 16 it is considered as juvenile rheumatoid arthritis (JRA). Occasionally several members of a family are affected by RA and in monozygotic twins there is a higher incidence that expected. Genetic predisposition has been observed in class II gene products (HLA-DR, DO, DP) of the major histocompatibility complex. The nucleotide sequence of the HLA-DR β1 exons encoding the 5 amino acid residues 70-74 predicts susceptibility to RA and is associated with RA in 83% Caucasian patients in the United King. RF are antibodies directed towards the Fc portion of IgG; It is found in 75-80% of patients with RA. High RF IgM titers are relatively specific for the diagnosis of RA (5).

RA is an inflammatory, chronic, degenerative disease of a systemic character whose etiology is multifactorial. Studies in relatives have shown that within the family there is a high degree of predisposition towards autoimmunity. Predisposing factors are: female sex, family history of RA, advanced age, exposure to silicates and smoking (6). Endocrine, metabolic and nutritional factors have been studied, as well as geographical and occupational variables. Polyarthritis occurs in humans during many bacterial, viral and spirochete infections. In patients with RA it has been observed in virus infections, mycoplasma; parvovirus has been found in the joints with negative rheumatoid factor (RF). The observation of high titers of serum antibodies against the Epstein Barr virus (EBV) in patients with RA reflects an abnormal response towards this pathogen. The disease characteristically begins in the small joints of the hands, and feet and progresses in a centripetal and symmetric fashion. Extra-articular manifestations include vasculitis, atrophy of the skin and muscle, subcutaneous nodules, lymphadenopathy, splenomegaly and leukopenia.

The genetic mechanism that promotes the development of RA is unknown; but HLA-Dw4 may import a genetic susceptibility to an environmental factor, such as a virus, that initiates the diseases process. Some studies have suggested a possible relationship between EBV and RA (7). Theoretically immune complexes initiate vascular inflammation by the activation of complement. A interesting finding in the joint effusion of patients with active RA is an elevation of B2-microglobulin levels.

RHEUMATOID FACTORS

RF are antibodies against antigenic determinants; are immunoglobulins of any isotype with antibody activity directed toward antigenic sites on the Fc region of human or animal IgG, these are found in 75-80% of RA patients. Most laboratory technics detect 19S IgM rheumatoid factor, but RF properties are also seen in 7S

IgM, IgG and IgA immunoglobulins. The most frequent isotype is IgM; is increased in autoimmune and non autoimmune diseases.. Especially those with the HLA -DR4 haplotype. High RF titers are generally associated with more severe and active joint disease; the presence of nodules, systemic complications and a worse prognosis (8).

RF is not specific for RA; it is detected in other autoimmune diseases including Multiple Sclerosis, Systemic Lupus Erythematosus (SLE), vasculitis and idiopathic pulmonary fibrosis. The prevalence of RA is estimated to be about 0.5 – 1% in North American and European caucasians over the age of 15 years. Patients commonly present to the clinician with a history of bilaterally symmetrical polyarthritis affecting at least 3 or more joints and morning stiffness lasting at least one hour. The joints most commonly affected are those of the hands, wrists, elbows, knees, ankles and feet.

DIAGNOSIS

The incidence in women occurs between 40 and 60 years of age. In Mexico, in the province of Yucatan, a prevalence of 2.8% was found. There are 3 basic pathologic findings:The first is a recurrent or chronic inflammatory of the joints leading to an inflammatory thickening of the synovium called the "pannus". The second finding the rheumatoid nodule consists of an irregularly shaped central zone of fibrinoid necrosis surrounded by a margin of large mononuclearcells. The third finding the presence of vasculitis

The first assay employed for the detection of autoimmune diseases came from the observation of Waaler in 1940 that sheep red blood cells sensitized with rabbit anti-sheep red blood cells which are agglutinated in sera of patients with RA. He named the factor responsible for the agglutination rheumatoid factor . RF was later determined to be an antibody reacting with the constant region of IgG. By 1957 various investigators found a specific interaction of DNA with the sera from patients with SLE. They demonstrated that the reactivity was due to an immunoglobulin.

Numerous antibodies were associated with RA but none were very sensitive or applicable to widespread clinical use. But in 1998 were found that many RA autoantibodies detect citrullinated proteins and that the antibodies are present early in disease. The common feature of these antibodies is that all react with proteins that are citrullinated. Antibodies to perinuclear factor were first described in 1964 in patients with RA. The presence of antibodies to keratin in RA

patients was described in 1979. The sensitivity for RA varies from 36 to 59% and the specificity from 88-99%. Later was observed that profilaggrin is postranslationally modified in two ways. It is proteolytically cleaved during cellular differentiation to become filaggrin and approximately 20% of the arginine residues are converted to citrulline. The risk of developing RA and the presence of positive anti-CCP antibodies is 20 times higher for smoking patients. (6,9)

LABORATORY FINDINGS

Normochromic or hypochromic normocytic anemia is observed as in SLE. Low iron levels are occasionally observed, however anemia is resistant to iron therapy. The total leukocyte count and the differential is generally normal, eosinophilia may be present in the presence of vasculitis. High RF titers occur in the presence of rheumatoid nodules. A small percentage of patients have anti-DNA antibodies (generally single chain) and other nuclear antigens. There is evidence to suggest that HLA-DR4 is associated with a more aggressive form of the disease. Synovial fluid shows a leukocyte count of 5000 to 20000/ml with 50-70% polymorphonuclear cells. Complement

levels are low in synovial fluid and there is evidence of hyaluronic acid degradation. One-fourth of patients have lymphocytosis. The sedimentation rate is elevated. The synovial fluid is more inflammatory that seen in osteoarthritis or SLE.

IMMUNOLOGIC DIAGNOSIS

The differential diagnosis between RA, SLE, scleroderma and rheumatic fever is sometimes difficult. In RA, serum protein electrophoresis may show an increased α2-globulin, a polyclonal hypergammaglobulinemia and hypoalbuminemia. Serum complement levels are usually normal. Five to 10% of patients have a false positive VDRL. 8-27% have a positive LE cells and 20-50% have antinuclear antibodies (ANA). Rheumatoid factor is present in over 75% of patients. It is important to emphasize that a negative RF by routine laboratory procedure, does not exclude the diagnosis of RA. RF is also present in patients with SLE 30%, in a high percentage (90%) of patients with Sjogrens syndrome. In the patients with classic articular changes, bony erosions of the small joints of the hands and feet and RF in serum or synovial fluid, the diagnosis of RA is not difficult. Patients with SLE, can be distinguished by their characteristic skin lesion, renal disease and diagnostic serologic abnormalities (See table 1).

A large variety of autoantibodies have been described in the serum of RA patients. Among these are: **Factor Rheumatoid**; the most frequent isotope is IgM, it is increased in autoimmune and non-autoimmune diseases. **Antinuclear antibodies (ANA)**. These are found between 10 to 50% in RA patients They generally present a homogeneous pattern, most are antihistone. The presence of ANA has been linked to severe erosive disease; in addition ANA can be included by drugs, including anti-TNF therapies. **Anti Ro/SSA and anti-RANA** (rheumatoid antigen nuclear) antibodies have also been found. **Anti-phospholipid** antibodies have been described but have not been related to RA. **Type II anti-collagen** antibodies; their importance has been questioned become they also appear in SLE and psoriatic arthritis. In RA are detected in serum, synovial fluid and cartilage between 53 to 60% of patients.

Anti-RA-33: they are specific antibodies for the nuclear heterogeneous ribonucleoprotein; have a sensitivity of 26 to 28% and these disappear when there is remission.(10)

SPECIFIC ANTIBODIES TO RA

Anti-glucose-6-phosphate isomerase. Is an enzyme that participates in glycolysis. This antibody occurs in 64% of patients with RA in serum and SF.

Anti-Sa antibodies. Sa is a protein present in the placenta, spleen and joint synovial tissue of patients with RA. These antibodies have a sensitivity of 40% with a specificity of 99% in serum and SF of patients with RA.. **Sa** is a hapten transporter antigen where vimentin is the transporter and citrulline is the hapten. On the other hand citrullination of vimentin is closely related to apoptosis due to the action of caspases (11).

Anti-keratin (AKA), antiperinuclear factor (APF) or antifilaggrin antibodies.. AKAs are antibodies directed against the stratum corneum of the rat esophagus. These have a sensitivity in RA of 29 to 55% and their specificity is 95-100%. They occur together with elevated PCR, ESR and RF values. APFs react against an antigen present in the keratohyalinized granules of the cytoplasm of cells of the human buccal mucosa. Their sensitivity is 86% with a specificity of 99%. Both types of antibodies react against the same antigen both recognize epitopes of the filaggrin protein of epithelial cells.

Filaggrin is produced during the last phases of the differentiation of epithelial cells in mammals. AKA, AFP and antifilaggrin antibodies recognize several epitopes it has been shown that regardless of the epitope recognized by these antibodies in all of them there is always an amino acid residue citrulline (12).

Citrullinated cyclic anti-peptide (CCP) antibodies. Citrulline is an amino acid that results from the action of the enzyme peptidyl-arginino-deaminase (PAD) on arginine. The activity of the enzyme is regulated by estrogens, which indicates its pathogenic role and the high prevalence of RA in females. (12). Several citrullinated proteins have been described in mammalian cells: myelin basic protein, filaggrin, trichohyalin, vimentin (which reacts with anti-Sa antibodies). histones, and fibrinogen. The importance of anti-CCP antibodies stems from their early appearance; there is data that indicates that it occurs up to 10 years before the first symptoms of RA appear.

Anti-CCP antibodies are currently used to diagnose the disease; these antibodies recognize peptides that contain citrulline which is a modification of arginine and corresponds to an amino acid that is not incorporated into the polypeptide chain during the synthesis process. Thus the antigenic target of these Anti-CCPs are the citrullinated peptide residues of filaggrin (13). In most cases of RA the clinical symptoms are initially mild and nonspecific, and a good number of patients do not complete the criteria for the classification of RA. Anti-CCPs can be detected 3 to 6 months before the first symptoms appear. The specificity on anti-CCP antibodies is approximately 95-98% with respect to undifferentiated forms of

arthritis that do not develop RA.

JUVENILE RHEUMATOID ARTHRITIS (JRA)

It is the most frequent disease in childhood and the one that causes the greatest degree of disability. Its etiology is unknown, the parvovirus B19 virus, rubella virus and EBV have been involved. Its distribution is universal and affects all races, it can be RF positive with ANA up to 70% positive. Major immunologic features**:** negative or " hidden " RF in the serum and synovial fluid. Elevated serum and depressed synovial complement. JRA is probably a group of disorders that cause crippling arthritis in individuals under 16 years of age. Some of these children have ankylosing spondylitis, others may eventually develop SLE, RA, psoriasis or chronic inflammatory bowel disease, while most never develop another specific disease. Systemic manifestations include fever, erythematosus rashes, nodules and leukocytosis, pleuritis, pericarditis and nephritis occur with less frequency. The disease affects predominantly girls, most between 5 to 12 years of age at onset. RF of all immunoglobulin classes have been detected.

Approximately 20% of children with JRA have a positive latex fixation test for 19S IgM RF. Cryoglobulins are associated with severe disease. CH50, C3 and C4 in the synovial fluid tend to be low; particularly in patients with a positive serum latex fixation or with IgG RF in the synovial fluid.

Laboratory findings. The synovial injury of JRA is a nonspecific synovitis with increased vascularity, infiltration of synovial fluid with lymphocytes, plasma cells, and macrophages, osteoporosis and periostitis. A normochromic microcytic anemia in the range of 8 – 11 g/dl hemoglobin is common and a mild leukocytosis between 15000 – 25000/ul is the rule. These patients have an elevated erythrocyte sedimentation rate and abnormal C reactve protein, Thirty to 50% of children have elevated ASO titers. Abnormal serological finding include the presence of RF in 10 to 20% of patients., ANA in 13% of patients and occasionally the LE cell phenomenon.

In order to determine the predictive value of anti-CCP antibodies in patients with early arthritis, 273 RA patients who had diseases symptoms of less than a year were evaluated over 3 years and 6 years periods in terms of physical disability, radiologic damage and the presence of anti-CCP. The results indicated that 70% of patients have anti-CCP early in the disease. With anti-CCP developed significantly more severe radiologic damage than those without anti-CCP. (14)

IMMUNOLOGIC PATHOGENESIS

There are two simultaneous Immunological processes that can explain the inflammation and tissue destruction characteristic of RA. The first process observed in the synovial fluid, results from the interaction of antigens and antibodies in the joint tissues and involves the complement sequence, observing low levels of this, increased vascular permeability and accumulation of cellular elements from the blood. In the rheumatoid synovial membrane there is an abundance of macrophages and cells with long and spiny dendritic processes; these cells function as antigen –presenting cells and can make large amounts of interleukins. Chronic RA is characterized by the destruction of joint cartilage, ligaments, tendons and bone. Collagen resists degradation by nonspecific proteases when in its triple helical configuration. RA affects the wrists; synovial proliferation on the palmar aspect can compresses the median nerve and cause carpal tunnel syndrome.

Citrulline is a modification of arginine that is generated by the action of the enzyme peptidylarginine deaminase (PAD) that converts peptidylarginine into peptidylcitrulline. Five isotypes of this enzyme have been identified, distributed in different tissues: PAD I is mainly expressed in the epidermis and the female reproductive system, PAD II in skeletal muscle, spleen, brain and secretory glands, PAD III in hair follicles, PAD IV in neutrophils and skeletal muscle, PAD V in embryos and zygotes. In citrullination, physicochemical processes occur that lead to the loss of the positive charge of arginine towards a neutral charge of citrulline and causes changes in the primary, secondary and tertiary structure of proteins that affects the ability of peptides to covalently bind to HLA molecules. This charge is recognized by the immune system generating antibodies. Anti-CCPs have a specificity of 98-99% as serological markers for RA and a sensitivity of 80% (15). Several citrullinated proteins with high specificity for RA have been described. Among them are filaggrin, type I and II collagen, fibrinogen and vimentin. The latter has been found in the synovial fluid of patients with RA (16). Three citrullinated protein have been described in mammalian cells: myelin basic protein, filaggrin and trichohyalin; however also other proteins such as vimentin and histones undergo citrullination during programmed cell death of leukocytes- Anti—CCPs can appear up to 10 years before the first symptoms of RA appear.

Interleukin IL-17 has been implicated in the development of various autoimmune diseases, among them RA stands out, since it has been found in affected areas. In addition, IL-17 stimulates the differentiation of osteoclasts and promotes the destruction of cartilage and bone. The prevalence of RA in first-degree relatives is higher than in the general population; has a 12-15% agreement. Heritability in RA has been estimated to be 60-70%. Several genes that participate in the innate and adaptive immune response have been associated with the pathogenesis of RA. These include some HLA class I, II and III alleles, cytokines, chemokines, adhesion molecules and metalloproteases (17). On the other hand, NAT_2 slow acetylator genotype may be a risk factor of individual susceptibility to RA. The risk of development of RA was almost 5 fold greater in slow acetylators that in fast acetylators (18).

PATHOPHYSIOLOGY

Interleukins play a key role in integrating responses to a variety of stimuli in inflammatory processes. Some cytokines such as interleukin IL-1, IL-6 and tumor necrosis factor (TNFα) are pro—inflammatory. Tumor necrosis factor (TNFα) is probably the most important cytokine in RA. This protein is produced by the TNFα gene, which is found in the 6p21 cytogenetic band. This factor has been shown that TNF, IL-18 and IL-6 can induce the development of RA.(see figure 1) IL-6 interleukin in 50% of patients, its values were 6 times higher than normal subjects. (19).

On the other hand it is suggested that some amino acids can be considered as a markers for diagnosis of RA and monitoring pharmacotherapy of the disease (20). RA is characterized by chronic inflammation of the synovial joints with synovial proliferation and infiltration by blood derived cells, memory T lymphocytes, macrophages and plasma cells. Synovial tissue becomes hyperplastic and invasive at the interface of cartilage and bone with the progressive destruction of these tissues in most cases. Activated T cells contribute to the regulation of osteoclast activation and joint destruction. B cells also play an important role in the pathogenesis of RA; these cells generate immunoglobulins, RF and other autoantibodies. (21)

The series of microorganisms resident in the gut are termed microbiota; which is considered to play an important role in the pathogenesis of autoimmune diseases, including those outside the gut. (22). The production of metabolites from the microbiota can be pro-inflammatory and to be initiators of the disease or may serve as immune regulators. Relevant compounds produced by the microbiota for the maintenance of immune homeostasis include: short chain fatty acids, tryptophan metabolites, polyamines and others (23). Regarding tryptophan metabolites the gut microbiota can produce several molecules; these include indole-3-aldehyde, indole-3-acetic, 3-methylindole and indole-3 lactic acid. In a rat model of spondyloarthritis, administration of short-chain fatty acids attenuated the disease and up regulated the production of spermidine in cecal contents (24).

Over the evolution, some amino acid metabolic pathways have indeed become clinical check points for controlling adaptive immune responses to self and exaggerated inflammatory outcomes. (25). Each degradative pathway is characterized by a rate-limiting enzyme, whose expression is normally subjected to strict regulation. The most important enzymes are indoleamine 2,3-dioxigenase1 (IDO 1) and arginase 1 (Arg 1) which limit the catalytic rate in L-tryptophan and L-arginine metabolisms respectively, and ornithine decarboxylase 1 (ODC1), which produces polyamines (26). An important source of amino acid metabolites (not always produced by mammals) is also the microbiota, consisting of a wide variety of bacteria, viruses, fungi and other microorganisms that inhabit the human body in health and disease. In fact, gut bacteria produce tryptophan metabolites that attenuate inflammation in the host. Because an altered composition of gut microbiota has been observed in RA patients the co-metabolism of amino acids by symbiotic microorganisms and the host may also be subjected to

relevant modifications and pathogenetic consequences (27)

Recently has been demonstrated that the oral and gut microbiomes are perturbed in RA and partly normalized after treatment. In a recent study, butyrate (a short-chain fatty acid) supplementation reduced experimental arthritis severity via an increase in 5-hydroxy indole-3-acetic acid, a tryptophan metabolite derived from serotonin and agonist of aryl hydrocarbon receptor (AhR) (20). The activation of such a mechanism promoted the differentiation of B lymphocytes into regulatory cells. .These data demonstrated that supplementing the diet with certain microbiota-derived molecules, which directly or indirectly favors AhR activation by tryptophan metabolites, may be a promising treatment for RA. (28)

Tryptophan metabolism

Tryptophan is an essential amino acid precursor for protein synthesis and the generation of several molecules involved in fundamental biological process (29). The 99% of tryptophan is metabolized along the kynurenine pathway and the remaining 1% is converted in serotonin and melatonin. The kynurenine pathway is initiated by the transformation of tryptophan into N-formylkinurenine that is rapidly converted into L-kynurenine. An important metabolite of tryptophan is nicotinic acid; the final product of the pathway is the nicotinamide adenine dinucleotide (NAD+) cofactor, which has a fundamental role in redox reactions that are essential for mitochondrial functions.

Several data have been reported for a possible association of IDO I with RA pathogenesis. In sera of RA patients, tryptophan concentrations were found to be reduced. On the other hand, synovial tissues of RA patients are characterized by hypoxia and hypoxic conditions are capable of reducing IDO I expression, tryptophan metabolism and T cell -supressive capacities of synovial fibroblast.

Serotonin. The enzyme tryptophan hydroxylase converts tryptophan amino acid into 5-hydroxy-tryptamine, which is then sequentially converted to serotonin. Serotonin is a biogenic amine. In RA patients, increased levels of circulating serotonin have been reported; serotonin levels are altered not only in peripheral tissues but also in synovial fluid, the brain specially in the hippocampus. N-actetylserotonin (NAS) is produced from serotonin by the arylalkilamine N-acetyltransferase enzyme; NAS has recently been shown to exert significant anti-inflammatory and protective effects in a mouse experimental model of multiple sclerosis, a chronic inflammatory/autoimmune disease.(30)

Melatonin. Melatonin is synthesized mainly in the pineal glands but also in brain, gastrointestinal tract, bone marrow, lymphocytes and skin. Melatonin is beneficial in several inflammatory autoimmune diseases, including SLE, MS, inflammatory bowel disease and type 1 diabetes. However, its effects in RA remain controversial. In pinealectomized mice (level of melatonin are reduced a 70% in sera) showed a reduced severity of arthritis. Interestingly melatonin administration increased the severity of arthritis by decreasing the expression of cryptochrome 1 (Cry 1), a circadian clock gene and by increasing serum concentrations of IL-6 and TNFα, two pro-inflammatory cytokines. In RA patients, melatonin levels in sera were found to be significantly higher than healthy subjects and these levels correlated positively with disease activity scores and the erythrocyte sedimentation rate (ESR). Moreover, in the early morning when melatonin levels are higher, patients with RA exhibited high serum concentrations of pro-inflammatory cytokines, especially TNFα and IL-6 (20, 31)

L-ARGININE

Arginine is an amino acid considered important of the immune response and has high chances of being implicated in the molecular mechanism of RA pathogenesis as outlined below. Arginine can be catalyzed by 5 different groups of enzymes; arginase I, and arginase II as part of the urea cycle, nitric-oxide-synthetase, arginine-descarboxilase and arginine-glycine-amidinotransferase, through these processes arginine gives rise to ornithine, urea, polyamines, proline, nitric oxide (NO), citrulline, proteins, glutamic acid, agmatine and finally creatine. Arginine also regulates the activation of macrophages and T lymphocytes.(32).

L-arginine is degraded by two major families of enzymes: nitric oxide synthases (NOS) and arginases. NOS catalyzes the conversion of L-arginine into NO and citrulline, which is recycled back into L-arginine by the sequential action of argininosuccinate lyase (ASL) and argininosuccinate synthetase (ASS). Ornithine acts as a substrate of ornithine descarboxilase (ODC), ornithine aminotransferase (OAT) and ornithine transcarbamilase (OTC) to yield putrescine, proline and L-citrulline respectively (33).

Several investigations have reported significant alterations of arginase enzymes in RA patients. In one study, arginase activity was measured in synovial fluid cells from patients with different forms of arthritis, RA, osteoarthritis, psoriasis, arthralgia and juvenile chronic arthritis. Increased of ARG2 inhibited the production of NO by substrate competition.(34).

Overexpression of ARG2 inhibited the production of NO by substrate competition. In another study, arginase activity was measured in the plasma of RA patients. The results showed an increased level of plasma arginase activity, as well the decreased arginine bioavailability. Because increased arginase activity reduces the production of the vasodilator NO, dysregulated arginine metabolism may represent a risk factor for cardiovascular diseases, whose incidence is indeed increased in RA patients (35).

Polyamines. Polyamines, such as putrescine, spermidine and spermine are polycationic molecules present in almost all living cells and are integral to a wide range of biological functions including cell growth and death. Polyamines have ability to bind negatively charged macromolecules i.e. nucleic acids, proteins and phospholipids. L-arginine is a precursor of the polyamines, producing ornithine and this by action of the ornithine descarboxilase produces putrescine, which originates spermidine and spermine. RA patients have been found to accumulate spermine and spermidine in synovial tissue, synovial fluid and urine. (36). Spermine participates in the regulation of protein binding to RNA and interaction with DNA to protect it from denaturation and ionizing agents such as radiation from sunlight.

HISTIDINE

Plasma contains a high level of a histidine-rich glycoprotein, which has a multidomain structure, interacts with many ligands and regulates a number of biological processes, including cell adhesion and migration, complement activation, and phagocytosis of apoptotic cells (37). Histidine is a basic amino acid whose deficiency causes some types of anemia because it stimulates the production of red blood cells. It is an essential amino acid for some enzymes that contain metals in their structure such as superoxide dismutase (metalloprotease).In clinical studies carried out in patients with RA, low serum levels have been found with respect to healthy subjects. This is also observed in arthritic synovial fluid (38). When histidine is administered to patients with RA, the symptoms of the disease improve. All RA patients who received histidine had a better ability to walk and decreased joint inflammation. (29) Some medications such as gold salts, chloroquine and D-penicillamine keep serum histidine levels within normal ranges. On the other hand, it was observed that serum histidine levels decrease when taking conventional anti-inflammatory drugs even though they are taking histidine; this may be due to the fact that some NSAIDs inhibit histidine and ornithine descarboxilase altering the metabolism of histidine and also the synthesis of collagen; which is likely to cause deformation of the finger and other joints. (39) Histidine participates in the oxygen transport function of hemoglobin and is necessary for the synthesis of hemoglobin. The heme group is linked to globin by a bound between the iron atom and a nitrogen atom contained in histidine β92 (F8) or histidine α87 (F8). Patients with elevated sedimentation rate (ESR) and difficulty walking are those who respond best to histidine treatment. (40).

Histidine is essential for hemoglobin synthesis; furthermore, a histidine deficient diet is associated with anemia development; this is because iron does not bind to globin; therefore. RA patients do not improve with the administration of iron. Anemia associated with decreased histidine concentration in the blood has been repeatedly observed in patients with RA. (41).

HOMOARGININE

L-Homoarginine (HA) which is present in **Lens culinaris** (lentils), **Lathyrus cicero** and **lathyrus sativus** differs from L-arginine only in that its contains an additional backbone methylene group (CH3) and can replace L-arginine in mammals in most physiological processes. Arginine has a short half life due to the pancreatic arginase; but this enzyme does not act on HA and thus HA circulates longer in the blood and may act as a better insulin secretagouge; also is a good inhibitor of

glycine uptake at the synapse. (42). The amino acid HA is a metabolite of ornithine in human metabolism and mammalians. Homocitrulline and homoarginine, both amino acids can be detected in larger amounts in urine or blood of individuals with urea cycle disorders (43),

Homocitrulline has been identified as an antigen specific rheumatoid arthritis as a target of anticitrulline protein/peptide antibodies. More recently, it has been shown that homocitrulline-containing proteins are present in rheumatoid arthritis joints of rodents and that they may affect T-cells triggering and possibly can modify protein structures and ultimately cause metabolic dysfunctions.(see figure 1). Interestingly, antibodies to citrullinated proteins are sometimes present in the blood of subjects who later develop RA, years before the clinical onset of the disease. The presence of homocitrulline in proteins is also a frequent finding in inflammatory conditions. Antibodies binding to homocitrulline have been found in RA patients sera and also are present in RA joints. (44).

A study of the free amino acids and related compounds in the seeds of 49 species of **Lathyrus** showed the presence HA a non protein amino acid. Homoarginine was isolated from **L cicero** and **L sativus**; and was a substrate for rat liver arginase. This enzyme catalyzed the hydrolysis of HA to yield urea and lysine. When HA was fed to rats on a lysine deficient diet food intake any growth rate were both reduced. The concentrations of ornithine, lysine and arginine in the brain were decreased. Homoarginine is an uncompetitive inhibitor of human liver and brain alkaline phosphatases, but is not active against the intestinal and placental forms of the enzyme, furthermore does inhibit the uptake of arginine by vascular endothelial cells. (45).

Though arginine was initially recognized as the physiological substrate for NO generation, it is now recognized that it can be generated from HA also. HA is in fact a better substrate for NOs since it results in a sustained NO generation. (46).

CANAVANINE

L-canavanine is a substrate for the arginase enzyme and when is metabolized it produces urea and canaline (an analogue of ornithine). Canavanine is an amino acid that is not part of animal proteins, but because of its structural similarity to arginine, it takes its place and therefore affects the metabolism of arginine.(see figure 1) The toxicity of canavanine is due to the fact that inhibits protein synthesis. Arginine is an important constituent of histones; the substitution of arginine by canavanine decreases the cationic charges of the proteins; therefore this is recognized by the immune system generating anti-histone antibodies. Levels of canavanine in serum were evaluated in RA patients; 60% of the patients presented high values compared to healthy subjects. (47) This coincides with ANA values found in RA patients. Canavanine can affect B-cells function, accelerating the disease and also affects the charge surface membrane properties of autoimmune B-cells. Such alterations may be associated with an abnormal immune response. It is possible that homoarginine together with canavanine are a trigger of RA since the two amino acids alter the immune response; thus canavanine generates ANA leading to the development of SLE when consumed for a long time and homoarginine generates citrullinated proteins. (48). Lentils contain HA and several antinutrients that are trypsin inhibitors an enzyme that binds iron and zinc, moreover contain 1g% dry weight canavanine (49).

TREATMENT

DRUG TREATMENT

1. Salicylates are the main stay of medical therapy of RA. Aspirin is an anti-inflammatory as well as an antipyretic and analgesic agent. Several other nonsteroid anti-inflammatory agents (NSAIDS) have been used in the treatment of RA. Indomethacin and naproxen may be useful in patients who cannot tolerate aspirin. If patients do not respond to NSAIDs and other forms of conservative therapy, slow-acting medications such as gold salts, antimalarials, penicillamine, and methotrexate should be considered. These agents can cause improvement for variable periods, but their long-term impact on the course of the disease has not been documented. (50)
2. Gold salt therapy. Parenteral gold salt therapy is of significant benefit to many patients. It is one of the few therapeutic agents that is believed to alter the long-term course of the disease. Gold salts are used because they are able to suppress joint inflammation; the disadvantage is that they produce eosinophilia, thrombocytopenia and leukopenia. Toxic side effects occur in 40% of patients and include dermatitis, photosensitivity, stomatitis and nephrotic syndrome.
3. Penicillamine has been found to be useful in the treatment of RA, because there is a significant improvement compared to placebo and it is effective in many patients. The mechanism of action is unknown but it appears to lead to slow improvement, requiring up to 12 months to achieve máximum clinical benefit.
4. Methotrexate. Currently methotrexate is the standard treatment, it is used as a base drug to accompany treatment with biological agents. Methotrexate is a folic acid analog and is widely used for the treatment of RA; its toxicity is less than that of other medications because it is administered as weekly pulse therapy. Some people taking methotrexate develop nodules on the feet and hands. Bone marrow suppression and hepatotoxicity are rare complications. (51).
5. Corticosteroids. Corticosteroids is useful if the patient has a limited number of symptomatic joints. Systemic corticosteroids may induce a dramatic clinical response but do not alter the course of the disease.

There is evidence that some pharmaceutical drugs used in the treatment of rheumatic diseases affect NO activity. Auronofin , glucocorticoids and cyclosporine inhibit the induction of NOs in various tissues. Tetracyclines inhibit NO production in chondrocytes and macrophages, also aspirin, diclofenac and tenidap inhibit NO synthesis. (52).

Approximately 50% of patients have deformities. These deformations before any arthropathy should be thought that is the undesirable effect of a drug. People who smoke may be more vulnerable to developing rheumatoid nodules; nodules in RA patients are formed by altered collagen. Most of the NSAIDS are organic acids with low pKa values, capable of accumulation in the inflamed tissues, characterized by acidic pH. (53) There are several drugs that can cause arthralgia symptoms; among them are antibiotics, antihistamines . The mechanism is thought to be a blockage of H_2 receptors in the joints. The drug that has been most frequently associated with RA is metoprolol; also laxatives when consumption is frequent (54).

FILAGGRIN

Filaggrin is a filament protein that had been isolated from the stratum corneum. The higher concentration of calcium in the stratum granulosum causes the keratohyalin granules to release their contents, leaving the profilaggrin exposed to undergo processing and fragmentation into active filaggrin monomers. This free filaggrin interacts with intermediate keratin filaments, causing their aggregation into macrofibrils and compaction, provoking the flattening of the cell. Human filaggrin is a histidine-rich protein that comprises between 10 and 12 tandemly filaggrin repeats, which are flanked on either side by two partial filaggrin repeats and by N and C terminal domains.

In humans, filaggrin contain 324 amino acids and contains a short linker region that is proteolytically cleaved during conversion of the profilaggrin (55). After the proteolytic processing of profilaggrin, the resultant filaggrin monomers are processed further into amino acids and their derivatives. During the final stages of terminal differentiation several key epidermal proteins, such as filaggrin, keratin and trichohyalin, undergo deamination. Deamination of filaggrin within the stratum corneum changes the net charge of filaggrin from basic to nearly neutral which disrupts the ionic interactions of the filaggrin-keratin association. Breakdown of filaggrin into hygroscopic free amino acids and their derivatives such as pyrrolidone-5-carboxylic acid is the major contributor to the natural moisturing factor that is produced within the stratum corneum. Trans-urocanic acid (UCA) is another key derivative of filaggrin degradation acid and is generated from free histidine by histidase. These acids are 2 of the main factors responsible for maintaining the acid pH of the stratum corneum, which is essential for its antimicrobial action and for its regulatory role on enzyme activity and physiologic desquamation.(40).

There are a large variety of autoantibodies that have been described in the sera of RA patients: antikeratin (AKA) have been largely demonstrated to be the most specific serological marker of RA because their diagnosis specificity was reported to be from 95 to 100%. (56) They are increased recognized as being a major diagnosis tool (57). AKA are possibly involved in the pathophysiology of RA because: 1. They are highly specific for the disease. 2. They are associated to the more active and severe forms. 3. They may appear at very early stages

and even before the clinical symptoms and 4. Their ratio to global IgG is increased in synovial membranes with regard to the serum or the synovial fluid and they are synthesized by plasmocytes of the rheumatoid "pannus".

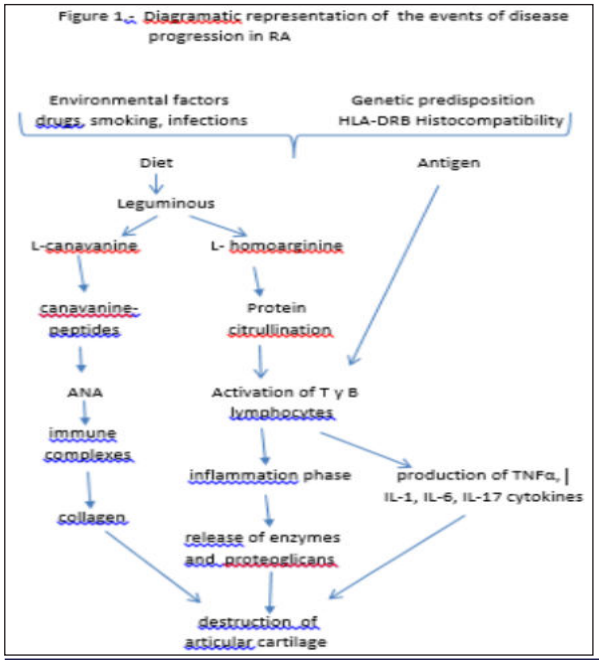
Currently early diagnosis is a great challenge for rheumatologists, because radiological signs and laboratory data, images and immunological alterations are not specific for this disease.. Anti-CCP antibodies are an essential tool in the treatment of RA. APFs and AKAs are now known as antifilaggrin antibodies as they react against native filaggrin. Titers of AFA are significantly higher in RA patients but not in patients with osteoarthritis, ankylosing spondylitis or SLE. AFA recognizes the uncitrullinated filaggrin and its titer correlates with clinical parameters. (58). CCP is detectable up to 10 years before the onset of the disease (56) . It has been observed that the progression of the disease in RA patients with positive CCP reaches up to 85% in 3 years. On the other hand, those who were negative CCP only 25% developed RA after 3 years. This fact indicates that treatment with pharmaceutical drugs can be started in patients with suspected RA but who still do not meet the conventional clinical but have positive CCP. It was observed that the group of healthy or sick controls but without RA, the PCC was only positive in 1 to 5%. On the other hand with the RF, the values obtained were from 10 to 30%. (59).

It is also hoped that increased understanding of filaggrin biochemistry and the citrullination process together with the clinical and histological information that will facilitate the development of novel therapeutic approaches and identity novel drug targets for these extremely common diseases.

Table 1.- Laboratory Finding Into Serum SLE And RA Patients

	SLE	RA
NNA	+	+
Leukopenia	+	+
Trombocytopenia	+	-
ESR	+	+
Complement levels	low	normal
Anti-dsDNA	+	-
Anti-ssDNA	-	+
VDRL	20%	10%
Anti-RA33	-	35%
ANTI-Ro (SSA)	-	40%
LE cells	70%	30%
ANA	93%	60%
Rheumatoid factor	30%	80%
Anti-CCP	15%	95%

NNA= normocytic normochromic anemia. ESR = elevated sedimentation rate



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