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Clinical Research

SYSTEMIC LUPUS ERYTHEMATOSUS. MOLECULAR BASIS OF DISEASE PART 2

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ABSTRACT The exact etiology of lupus erythematosus still is unknown. The genetic factors play an important role in the predisposition of the disease however some environmental factors are required to trigger the disease. Has been observed some relation among the activity of enzyme acetyltransferase and the presence of SLE; also has been found some disorders in structure of collagen during disease, so as changes of enzymes activity in blood and changes on levels of amino acids in blood and urine of patients with SLE. Among these amino acids are arginine, ornithine, proline and hydroxyproline. On the other hand also has been found non-protein amino acids on blood samples of patients with SLE and they are considered as triggers of SLE.

KEYWORDS : Molecular basis, SLE, canavanine, canaline, arginine, acetyltransferase

INTRODUCTION

The collagen diseases include rheumatoid arthritis, systemic lupus erythematosus (SLE), scleroderma and dermatomyositis. These diseases have three things in common; their etiologies remains unknown , the prognosis is not hopeless, remains poor and the specificity of available drugs questionable. The development of SLE is multifactorial depending on the genetics, susceptibility, consumption of pharmaceutical drugs, dietary habits, exposure to sunlight and environmental pollutants. Ultraviolet radiation is the environmental factor most linked to lupus and causes exacerbation in 70% of patients by increasing the apoptosis of the skin cells and disturb the DNA and the intracellular proteins become antigenic generating antibodies anti-chromatin and anti-phospholipid. On the other hand several drugs are able to induce a variant of lupus in which the dermatological and joint manifestation are frequent and the renal and neurological manifestations are rare. The genetic factor is important but it is not enough to cause the disease; the coincidence rate in monozygotic twins is approximately 25% and 2% in dizygotic twins. Lupus erythematosus is much more frequent in relatives of patients with SLE than in the general population. Population studies reveal that the susceptibility to SLE involves human leukocyte antigen (HLA) class II gene polymorphisms. The association of SLE with HLA antigens (HLA-DR2 and DR3) has been demonstrated in both white and black race, with a relative risk for the development of disease of approximately two to five. The HLA class II genes have also been associated the presence of certain auto-antibodies such as anti-Sm, anti-Ro, anti-RNP and anti-DNA antibodies. The exact etiology of SLE is unknown; the disease shows a strong familial aggregation with a much higher frequency among first degree relatives of patients. Moreover in extended families SLE may coexist with other organ specific autoimmune diseases such as hemolytic anemia, immune thrombocytopenic purpura and thyroiditis .

Role of ARGINASE

The collagen is a protein constituted by amino acids arranged in a specific sequence; the main amino acids are glycine (26%), proline (14.4%), hydroxyproline (12.8%); the last is almost exclusive to the collagen, for this reason the first amino acid to be investigated was hydroxyproline and following the biosynthesis route of proline; it was observed that proline is synthesized from ornithine; when this was evaluated, its blood levels were elevated in 62% of patients with SLE and the ANA titers were quantified observing that the levels of ornithine are also raised simultaneously. Ornithine is synthesized from arginine by the enzyme arginase; when arginase was evaluated was found in clinically healthy people from 5 to 17U/L and in patients with SLE the values were from 0.0 to 8 U/L (1). At first it was thought that serum arginase activity was increased in patients with SLE considering that ornithine levels are increased; however, the activity levels of the enzyme were low. The explanation for this fact is that the high concentrations of ornithine inhibit the activity of the enzyme. This biochemical process is well known in enzymatic reactions that when the product derived from the activity of the enzymes accumulates they are inhibited so that no more product is accumulated. The accumulation of ornithine is due to the fact that it is not metabolized because the enzymes ornithine-descarboxilase (ODC), ornithine-aminotransferase (OAT) and also ornithine-transamidinase are inhibited (2) . The consequences of the inhibition of the afore mentioned enzymes are: The collagen is synthesized in a deficient form because the enzyme peptidyl-lysyl-oxidase is also inhibited; therefore the peptides do not bind to form the complete chain of collagen protein and these small peptides are eliminated in the urine with an amount up to 4 times higher than normal. In the urine, free hydroxyproline and prolil-hydroxy-proline (60%) and glycyl-prolyl-hydroxy-proline (15%) peptides are excreted (3). The arginase enzyme in addition arginine, also catalyzes the hydrolysis of other compounds such as L-canavanine (a non protein amino acid present in alfalfa and edible beans), L-argininamide, L-homoarginine, arginine acid and agmatine (4); on the other hand enzymes ODC, OAT, ornithine-transcarbamilase (OTC), TGO, TGP, diamino-oxidase and others, are dependent on pyridoxal phosphate and are inhibited by L-canaline, cycloserine, hydroxylamine and thiosemicarbazide. It is well documented that the afore mentioned compounds increase the ornithine levels in addition to the following drugs that produce lupus erythematosus : methylthiouracil, levodopa, methyldopa and contraceptives (5).

The arginase activity in patients with SLE, 89% of them had values lower than 7 U/L; on the other hand, the titers of ANA were evaluated and was observed that the arginase activity correlate closely in an inversely proportional way. As the ANA titers increase, the arginase activity decrease in such a way that the titers of ANA 1:512 and more high the arginase activity is 0.0 U/L. Arginase activity begin to decrease with titers of ANA 1:128. The evaluation of arginase activity in patients with SLE should be done before administering steroids because these compounds increase the activity of arginase and others enzymes. Arginase is an enzyme found in almost all cells and tissues such as the liver, erythrocytes, leukocytes, kidney, saliva, prostate, etc. (6). Renal arginase transforms arginine into ornithine and is converted by means of the ODC enzyme dependent on pyridoxal phosphate into polyamines and is located mainly in the cytoplasm of the cells, spermidine 16%, putrescine 8% and spermine 17%. When activity of the arginase decreases, the synthesis of polyamines also decreases; therefore the growth and cell division also decrease and therefore decrease the production of erythrocytes (erythropenia), decreased leukocytes (leukopenia), decreased platelets (thrombocytopenia), decreased lymphocytes and decreased hemoglobin synthesis. All this is observed in the blood of patients with SLE; therefore they present normochromic normocytic anemia. There are two different isotypes of arginase I and arginase ll. Arginase l is found in the liver and accounts for 20% of the total conversion of

arginine to ornithine. Arginase I is also found in macrophages and endothelial cells. Arginase II is mitochondrial, found in kidney, brain, small intestine, mammary gland and macrophages. The type arginase II is the main one in the synthesis of ornithine as proline precursor and synthesis of polyamines. The activity of enzyme arginase has been evaluated in various diseases such as liver cirrhosis, hepatitis, obstructive jaundice, fatty liver, bruising, pneumonia, fever, metastasic cancer, iron deficiency, infections and in all of them normal or increased levels of the activity of arginase, therefore the evaluation of arginase in patients with SLE is considered very useful. In intestinal tissue it has been observed that the decrease of polyamines due to inhibition of ODC inhibits the proliferation of intestinal epithelial cells. When there is a hereditary deficiency of arginase, is observed hyper-argininemia, finding high concentrations of arginine in plasma, urine and CSF(7,8).

ARGININE

At present have been searched some amino acids that may be useful for diagnosis of the disease. When the arginine levels in blood of clinically healthy people were evaluated 4 to 17 mg/dl were found and in patients with SLE they were from 0.0 to 11 mg/dl of which 77% presented low levels of arginine. When comparing the levels of arginine with the ANA titers, it was observed that all patients with arginine levels less than 6 mg/dl have high titers of ANA; these values coincide with the activity of arginase (9). With the arginine values found, it was thought that several enzymes involved in the synthesis of collagen may be inhibited by some foreign compound or by an intermediate metabolite. On the other hand it has been observed that arginine is the main precursor of ornithine and when blood levels of ornithine are increased, this fact correlates with psychosis states, stress and with alterations at the immunological level. Several studies of immunological type have been made but they have not discovered the mechanism of formation of ANA that appear during the disease. When the lupus erythematosus syndrome associated with pharmacological drugs or chemical agents was observed; the first advances were made in the knowledge of SLE (10).

Arginine constitutes 8% of amino acids in the human collagen. The low levels of arginine found in patients with SLE is thought that it is also found in a lesser quantity in the collagen structure . A high content of arginine has been observed in anti-DNA antibodies in mice and patients with SLE; in addition, it has been observed that the pathogenicity of ANA correlates well with the content and location of arginine. Therefore the low levels of arginine found in blood of patients with SLE may be due to the fact that arginine is consumed for the formation of ANA. DNA contains arginine molecules at positions 94 and 98. When there is a change (mutation) of arginine at position 98 by lysine or methionine, no changes in attraction occur; instead a mutation in DNA position 94 by alanine is affected by the interaction with the protein molecules. Arginine at position 94 can bind DNA to form antibodies (11,12). The arginine is also an important constituent of histones, being approximately 15% of total amino acids. The modification in the amount of some amino acids and the substitution of arginine decreases the cationic charges of histones; therefore decreasing the interaction of histone with DNA (13). The liver synthesis citrulline and arginine is produced from it, most of it being converted into urea and ornithine. The kidney uses the citrulline produced by the liver to synthesize a greater amount of arginine by means of arginine-synthetase enzyme that is present in the renal medulla and is used for the synthesis of proteins in other tissues(14).

Serum levels of proline and hydroxyproline are not disturbed in patients with SLE; however in the urine the excretion of proline and hydroxyproline and glycine peptides are increased up to 4 times more than normal and their levels correlate well with activity of the disease. Proline is synthesized in the liver and kidney by deamination of ornithine that is an intermediate in the synthesis of arginine and is not incorporated into proteins; when its levels are increased, the enzyme arginase is inhibited. In laboratory rats; it has been observed that by administering arginine orally, the size of the thymus is increased, the blood levels of urea, creatinine, glucose and tests of liver function are not disturbed . On the other hand aggregated arginine in mixed cultures of lymphocytes, inhibits the development of cytotoxic T lymphocytes (15).

L-arginine is a precursor of the polyamines, producing ornithine and this by action of the ODC produces putrescine, which originates spermidine and spermine. These compounds bind to the clefts of DNA double strand. It has also been observed electrostatic bonds to the sugar-phosphate skeleton without altering the structure of DNA; on the contrary, they stabilize the double chain. 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 (for this fact, skin lesions appear due to sunlight**).** They also regulate the methylation of histones which is one of the main means of epigenetic control (due to the inhibition of ODC). Mammalian polyamines are synthesized intracellularly, are aliphatic cation; at physiological pH carry positive charge. The positive charge on the polyamines enables them to interact with DNA, RNA, phospholipids of the membrane and form complexes with it. Process like DNA replication, transcription and translation requires polyamines. In addition, polyamines increase polypeptide synthesis at the level of aminoacyl-tRNA binding to ribosomes. It was confirmed that polyamines exist mainly as a polyamine RNA complex in rat liver(16,17).

L-CANAVANINE a non-protein amino acid as trigger of SLE

In chimpanzees feed alfalfa, they developed hemolytic anemia and a disease with all the characteristics of lupus erythematosus. Afterwards, the association of SLE and the consumption of alfalfa was observed for the first time in a volunteer who developed lupus erythematosus while ingesting alfalfa seeds for a study of hypercholesterolemia. By suppressing the consumption of alfalfa the disease disappeared both in the monkeys and in the voluntary subject. The amino acid L-canavanina present in alfalfa was suspected of producing the disease. Subsequently several cases of induction or exacerbation of the disease due to the intake of alfalfa tablets were reported (18.). In rats fed a diet based on canavanine, alterations of the immune response were observed and the total development was affected as well as the growth of some organs and immune complexes were also deposited in the basal membrane of the renal glomerulus (19,20). Canavanine is a substrate for the arginase enzyme and when it is metabolized it produces urea and canaline (an analogous of ornithine). When canaline is injected into rats, renal failure occurs and high levels of ornithine are detected in the blood. Anti-DNA antibodies were found in the blood serum of chimpanzees and rats fed alfalfa and leukopenia and erythropenia were observed-. 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. When it was administered in rats canavanine marked with radio isotopes it was observed that the amount of canavanine that is incorporated in the proteins is equal to the amount of arginine. The substitution occurs in all the proteins that contain arginine altering the tertiary and quaternary structure of the proteins. The final result is an alteration of the enzymatic activity and a potential rapid degradation of the proteins which confers its toxic properties. The toxicity of canavanine is due to the fact that it inhibits protein synthesis and it has also been observed that the enzyme argynil-RNA-synthetase facilitates the incorporation of canavanine into nascent peptides chains and is disturbed the tertiary and quaternary structure(21). Canavanine is incorporated into the nucleus of cell and into the cytoplasm, it also interferes with the helix of DNA and RNA in formation. Canavanine can function in all enzymatic reactions in which arginine is a substrate; therefore canavanine can inhibit any enzymatic reaction that uses arginine as a substrate In a study performed in lysozyme in which canavanine is introduced, the enzyme loses approximately 50% of its catalytic activity. Canavanine inhibits DNA replication in kidney epithelial cells of monkeys. With radiolabeled canavanine 70% of the canavanine administered 24 horas in the proteins is incorporated in mice, 20 mg of canavanine were injected every hour for 24 hours . The DNA synthesis was reduced by 86% compared to the control level and up to 70% of canavanine is included in the proteins (22,). In addition the toxic effect of canavanine occurs when there is a high content of the amino acid in blood and when its ingestion is for a long time; so in monkeys fed alfalfa the disease appeared after 7 months and when the alfalfa intake was suppressed the symptoms of the disease disappeared . A voluntary subject of 59 years of age who daily ingested 80 grams of alfalfa seeds for 6 weeks; developing splenomegaly, leukopenia, positive coombs, hemolytic anemia, ANA and low complement; by suppressing the ingestion of seeds the spleen returned to its normal size and the other alterations returned to normal. There are several cases reported of patients with SLE that have been ingesting alfalfa seeds (23). This information suggests that the majority of patients with SLE have consumed alfalfa or some other legume that contains canavanine. The canavanine is found mainly in leguminous such as

alfalfa, soy beans, common beans, lentils and others (24).

Arginine is an important constituent of histones; they form the chromosomal structure and regulate the activity of the genes. The substitution of arginine by canavanine decreases the cationic charges of the protein; therefore the interaction of histone with DNA decreases and this is recognized by the immune system generating anti-histone antibodies(25). When the levels of canavanine in blood were evaluated in patients with SLE, were found 3.8 to 16 mg/dl and in healthy clinical subjects, their levels were 1.8 to 3.8 mg/dl (26). Figure 3. With these findings it is explained why the disease is up to 10 times more frequent in the families of patients with SLE and who live in the same house (27). This frequency of the disease is due to the fact they all consume the same foods among these are the leguminous. Leguminous are consumed since childhood and it has been observed that canavanine accumulates in the human organism causing illness in some people when they consume them for a long time. The consumption of canavanine together with the genetic predisposition for the appearance of the disease can be attributed to the differences of the acetylator genotype. (28). In the SLE produced by drugs, the dermatological and articulatory manifestations are frequent and on the other hand, the renal and neurological manifestations are rare. Generally the ANA titers correlate well with the activity of the disease. In a study done in 1010 subjects with SLE a sensitivity of 93% and specificity of 86% was found. Healthy people have ANA titers of 1:40 in 30% of subjects; 13% have titers of 1:80 and 5% have titers of 1:160 (29)(See table 1). On the other hand in patients who have anti-histone have found low levels of hepatic acetyltransferase. Some authors have proposed that anti-histone antibodies together with ANA are a marker highly specific in 98% to make the diagnosis of SLE and can be used for serological monitoring of SLE (25,30).

ARGININE METABOLISM

Arginine is an amino acid considered an important initiator of the immune response and serves as a precursor in several metabolic process in which arginine is converted to nitric oxide (NO) and ornithine and citrulline; it also regulates the activation of macrophages and T lymphocytes. Arginine is also produced from citrulline by the action of cytosol enzymes arginine-succinate-synthetase (ASS) and arginine-succinate-lyase (ASL), In addition, citrulline can be synthesized from ornithine by the ODC present in enterocytes and hepatocytes. This citrulline is released into the blood circulation, of which approximately 80% is taken up by the cells of the proximal tubule of the kidney for the synthesis of arginine (13,14). The consumption of arginine in the diet represents 40% of circulating citrulline. Arginine can be catalyzed by 5 different groups of enzymes: Arginase l and arginase ll as part of the urea cycle, nitric oxide-synthetase, arginine-descarboxilase (ADC) and arginine-glycine-amidinotransferase (AGAT). Through these processes arginine gives rise to ornithine, urea, polyamines, proline, NO, citrulline, proteins, glutamic acid, agmatine and finally creatine. About 15 to 20% of arginine enters at urea cycle where the enzyme arginase converts arginine to urea and ornithine (31,32). Arginine contains a hydrophobic chain and positively charged guanidine group. Histones contain approximately 15% arginine of all amino acids; they are highly cationic and their function is to fold DNA forming the chromosomal structure and regulate the activity of the genes. Arginine and lysine account for approximately 25% of the total amino acids that are part of histones**.**

CITRULLINATION or DEIMINATION

Citrulline is a modification of arginine and corresponds to an amino acid that is not incorporated into the polypeptide chain during the synthesis process; rather, it is generated by a post--translational modification of arginine by the action of peptidyl-arginine-deaminase (PAD), enzyme that converts the peptidyl-arginine into peptidyl-citrulline. The PAD enzyme acts on the arginine residues of the proteins and converts it into citrulline causing the loss of positive charge of arginine and therefore the interactions of the amino acid with its neighbors are modified. This modification favors the formation of antibodies. The enzymes called arginine deiminases (ADI) catalyse the deamination of free arginine, while the protein-arginine-deiminases (PAD) or peptidyl-arginine-deiminases displace the primary group ketamine (=NH) by a ketone group (=O). Arginine is positively charged at neutral pH while citrulline has not net charge. This increase the hydrophobicity of the protein which can produce changes in the protein folds affecting its tertiary structure and its function (33,34). The immune system can attack the citrullinated proteins, leading to autoimmune diseases such as rheumatoid arthritis and multiple sclerosis, psoriatic arthritis, SLE, Sjogrens syndrome and also in cells with Alsheimer´s disease; antibodies against peptide citrullinated are detected before the clinical symptoms of the disease appear. The trypsin protease normally breaks the positively charged arginine and lysine residues, whereas the trypsin is unable to break the citrulline residues which is neutral pH (35).

Rheumatoid arthritis is a disease that affects the synovial membranes of the joints; and like SLE is characterized by the intervention of genetic, environmental, ethnic, geographic and nutritional factors that lead to an autoimmune reaction. In citrullination the neutral charge of citrulline is recognized by the immune system when presented by HLA class II molecules. Several citrullinated proteins with high specificity for rheumatoid arthritis have been described, among which are fillagrin, collagen type I and II, fibrinogen and vimentin. The best known and used antibody is the rheumatoid factor which is an autoantibody directed against the Fc portion of the igG molecules. Antibodies against citrullinated cyclic peptide (CCPA) recently surged , which reach a specificity of 96% and sensitivity of 80%. In transgenic mice, peptide citrullination not only increases the affinity between peptide and the main histocompatibility complex, they also activate the TcD4 cells. Vimentin is a protein of two intermediate filaments of type III is found in all cells. CCPAs have proven to be a powerful biomarker that allows the diagnosis of rheumatoid arthritis in the early stages of the disease. These antibodies react with a series of citrullinated antigens such as fibrinogen and vimentin. CCPA is present in 20% of patients with SLE. Antigens that exhibit numerous variants with isoelectric points from 4.5 to 7.2 have been demonstrated in deaminized proteins. This autoantigen is an fillaggrin isoform and the presence of citrulline in myelin basic protein, multiple sclerosis with high concentrations of deaminized proteins due to PAD activity, has been described (34).

ANTINUCLEAR ANTIBODIES

SLE is considered to be prototype of autoimmune diseases in which the immune response is directed against a wide variety of self-antigens resulting in damage to various organs and systems. (See table 1). The incidence of the disease varies with ethnicity; in African-americans, Asians and Indo-americans it is 3 times higher and is more common in children of puberty age and more serious clinical features are presented with more organ involvement and worse prognosis than in adults. Antibodies against sDNA, ssDNA and nucleoprotein have been identified in extracts of postmortem renal tissue glomeruli from patients with SLE (36). Rheumatoid factor was also found; however to date, the structural basis of IgG auto-antibodies in human autoimmune disorders still remain poorly known despite all the attempts made with the new technologies. Anti-DNA antibodies represent an unique class of proteins that bind DNA of which there is little information. Single chain anti-DNA antibodies can bind to the puric or pyrimidic basis of DNA, to nucleosides. Double-chain anti-DNA antibodies can only bind to the polyribose-phosphate and to the base pairs guanosine-cytidine and adenosine-thymidine in their deoxi form (37,38).

Anti-DNA antibodies are the key to SLE; the IgG class of antibodies is included in the pathogenesis of the disease. Although the IgM class anti-ssDNA(single chain) may not be directly included in the pathogenesis of SLE. It has been shown that arginine and asparagine have an important role in the binding with DNA by anti-DNA antibodies (39). In addition, the number, location and topography of such residues are critical to confer anti-DNA specificity and avidity. Several research works have shown the sites and amino acids that participate in the interaction with DNA. According to these studies, the critical sites for DNA contact are in the 31-32 position in the HCDRl; positions 50-53 in the HCDR2, positions 96, 100 and 100A in the HCDR3; positions 29 and 31-34 in the light chain LCDR1; position 54 on the LCDR2 and position 92 on the LCDR3. The anti-DNA antibody sites are commonly occupied by basic and polar amino acids. Although these studies were done in murine models they are very important to understand the molecular basis of the anti-DNA-DNA interaction. (40,41)

Arginine is usually located on the surface of the proteins participating in the formation of salt bridges. Since 1950 in studies carried out both chemical, immunological and microbiological, it was established that canavanine (an analogue of arginine) is incorporated into the proteins in the place of arginine ´´in vitro¨ and ¨in vivo¨ replacing it completely

(22). The arginyl-tRNA synthetase enzyme incorporates the canavanine in the place arginine during protein synthesis, creating aberrant proteins, resulting in a potential and rapid degradation of proteins and can produce cellular apoptosis (42).

Lupus inducing drugs alter or weaken the histone-DNA interaction in chromosome domains since the drug linked to DNA has less ability to wrapping around the core protein, that does interleaved with DNA. When the formation of the nucleosome is affected by the DNA supercoiling, the possible unbinding of chromosomal domains by canavanine may influence the binding of the histones or the position of histone octamers in the core particles (43). This alters the binding with nucleosomes and can induce an immune response that results in the production of anti-histone antibodies and affects the arrangement of DNA-protein complexes in the nucleosome structure and may contribute to the presentation of structural antigenic epitopes that are contained in the chromatin histone-DNA complexes. Canavanine has an isoelectric point of 7.1 wherever arginine its isoelectric point is 12.48 which decreases basicity in histones that are rich in arginine; this alters DNA function and decreases histone synthesis; which has been demonstrated in mice, hamsters and monkey kidney cells (44).

Mice immunized with a polylysine peptide developed lupus disease, mice produce anti-sDNA antibodies and other antibodies characteristic of lupus including anti-histone, anti-cardiolipin and anti-ribonucleoprotein. At 3 months there were IgM and IgG deposits in the renal glomeruli of the mice. Anti-DNA antibodies in mice with lupus present an increase in the number of arginine residues in the VIICDR3 . This indicates that arginine contributes to the binding with ds-DNA during the formation of anti-DNA antibodies. In this way arginine contributes to the binding of DNA through the formation of ionic bonds with the negative charges of the skeleton of the phosphodiester of DNA. This is very significant because the pathogenicity of the antibodies correlates with the content of arginine in the anti-DNA antibodies. (45)

GENETIC and ACETYLTRANSFERASE

Although genetic factors and the hormonal milieu may create a predisposition towards SLE, the initiation of the disease probably results from several environmental triggers and exogenous factors. Infections agents may induce specific response by molecular mimicry and disturb immunoregulation; diet affects the production of inflammatory mediators, toxic/drugs modify cellular responsiveness, and immunogenicity of self- antigens and physical/chemicals agents, such as ultraviolet light can cause inflammation, induce cellular apoptosis and cause tissue damage. Many drugs such as procainamide and hydralazine which are aromatic amines or hydrazines can induce a lupus-like syndrome, especially in individuals who are genetically slow acetylators. In 1952 it was observed that isoniazid an antituberculous drug presents metabolic differences in different individuals and the main metabolites excreted in the urine are: acetyl-isoniazide, nicotinic acid and isoniazid. The excreted amounts of each compound vary in each individual. Later In 1964 it was established that the difference in acetylation of isoniazid is due to the enzyme acetyltransferase that presents polymorphism. In some individuals the half-life of isoniazid is 45 to 80 minutes (fast acetylators) and in others it was 140 to 200 minutes (slow acetylators). Acetyltransferase requires acetyl-CoA and is located in the liver and mucosa of the jejunum. Slow acetylators are recessive and fast acetylators are dominant. The acetylating phenotype of an individual is genetically predetermined. Individuals of Caucasian origin, African origin, from South India or from Mexico are slow acetylators. Individuals of Japanese, Chinese, Eskimal or Korean origin are fast acetylators. Slow acetylators have a high incidence of ANA and the appearance of SLE. The SLE is presented at 12 months in slow acetylators and in 4.5 years in rapid acetylators. In a study done by Drayer in 1977 of 134 patients, he found 104 that are slow acetylators. It has been observed that fast acetylators require a 58% more dose of anti-hypertensive to obtain the desired plasma values (28,46).

Advances in the knowledge of human genetics have allowed us to understand the function of genes, their allelic variants and gene-enviromental interactions (47). The existence of the genetic component in SLE is based mainly on family aggregation and high concordance in twins monocygotes it is estimated that the prevalence of SLE in relatives is 66 times higher than in general population. In the last years, several linked analyses have been performed in SLE and more than 60 potential susceptibility loci have been identified; of these approximately 52 genes associated with SLE have been reported. In the study of genes and their interaction in the response to a drug; it was discovered that the gene NAT2 (N-acetyltransferase) which presents polymorphism; this gene is located in the 8p22 region of the short arm of chromosome 8 and is made up of the non-coding exon 1 and the coding exon 2; the exonic region 2 is made up to 870 base pairs and 9 substitutions have been observed in its sequence or polymorphisms. These mutations affect the expression of the N-acetyltransferase enzyme by altering the speed with which it metabolizes its different substrates. In this way, three acetylating phenotypes are presented : fast, intermediate and slow.

The main function of N-acetyltransferase is to metabolize in the liver xenobiotics (drugs, foods and others) transferring from acetyl-CoA, acetyl groups to the terminal nitrogen of the xenobiotic generating new metabolites; which can be inactive or active molecules with biological effect. Other genes related to the genoma of SLE are the ITGAM gene involved in the activation of leukocytes, the adhesion of monocytes, macrophages and granulocytes. There is evidence that the substitution of arginine by a histidine alters the tertiary and quaternary structure of the interaction domain between ITGAM and its ligand and therefore modifies its affinity**.** The genes that are involved in the development of SLE are located on chromosomes 14 and 6; the first correspond to the gene of the histocompatibility class II; the second encodes for complement proteins and a third that affects immunoglobulin IIa and IIIa receptors (48).

THE COLLAGEN

The collagen molecule is an elegant structural that consists of three parallel polypeptide strands with a molecular weight of 300 000 daltons. At the ends of each chain, non-helical regions exist which are important in cross-linking and in the immunological specificity of collagen. The amino acid composition of the helical body of the chain is characteristic -every third residue consists of Gly, resulting in a repeating X-Y-Gly sequence, where X and Y can be any amino acid. The amino acids in the X and Y positions of collagen are often proline (28%) and 4-hydroxyproline (Hyp 38%) respectively; also contain hydroxylisine 0.5%. Prol-Hyp-Gly is the most common triplet (10.5%) in collagen. The carboxyl groups that exist in the collagen have a very important role in cross-links; the lysyl-oxidase enzyme responsible for cross-linking is also responsible for the oxidative deamination of lysine or hydroxylisine residues in the collagen molecule to form aldehydes with the amino groups of the neighboring collagen strands (49). The tensile strength of many tissues with a high content of collagen or elastin, is diminished after treatment with hydrazine because the degree of cross-linked collagen and elastin decreases. When the activity of the peptidyl-lysyl-oxidase enzyme that participates in the synthesis of collagen decreases; the precursor peptides of the collagen do not bind, and appear in the blood of patients with SLE and are eliminated in the urine in abundant quantity. The proline ingested in food is metabolized to $CO_2$and water, only 3% is used in the synthesis of collagen (46). The proline used in the production of collagen is synthesized from arginine and in the first reaction, participates the enzyme arginase producing ornithine. Collagen content in various tissues are: bone 88%, Achilles tendon 86%, skin 72%, cartilage 46 to 63%, cornea 68%, ligaments 27 to 75%, aorta 12 to 24%, elastin 26 to 32%. Of all known proteins, elastin has the highest concentration of glycine; it contains hydroxyproline but does not contain hydroxylysine. Hydroxyproline and hydroxylysine are virtually specific to collagen for which they form chemical fingerprints. Hydroxyproline stabilizes the molecule, in its absence, as in scurvy, the molecule is rapidly degraded in "vivo" (50). There are over 28 different types of collagen fibrils and are identified by Roman numeral designation. Types I, II and III collagens are the most abundant. Of these three major types of collagen, type I is by far the most abundant constituting nearly 90% of all collagen in the human body. Type IV collagen is a major component of all basement membrane; has a high incidence of 3-hydroxyproline in the X position (49).

Collagen subunits.

The collagen consists of 3 fractions called α, β and γ. Fraction **α** is the basic polypeptide unit which forms a dimer through covalent cross links fraction **β** originates and when it forms a trimer it constitutes the **γ** fraction. The common collagen of the skin is formed by two alpha I chains and one alpha 2 chain coiled to form a triple helix called the tropocollagen. These 3 units contain a high proportion of proline and hydroxyproline residues. In addition numerous modifications take

place to amino acids residues on the procollagen proteins; these modifications include hydroxylation and carbohydrate additions. Specific proline residues are hydroxylated by prolyl-3-hydroxylase and prolyl-4-hidroxilase. On the other hand has been observed that when the protein is synthesized on conditions that inactive the enzyme prolil-hidroxilase, this loss its native configuration, therefore is denatured to 34°C, while the normal collagen is denatured to 39°C. The cofactors of these enzymes are molecular oxygen, ferrous iron, ascorbic acid, and α-cetoglutarato. The next step is glycosylation of specific hydroxilisine residues by galactosyl or glucosyl transferase both of which are manganese dependent. Following completion of the processing within the Endoplasmic Reticulum and Golgi complex; procollagen proteins are secreted into the extracellular space, several reactions take place within the extracellular compartment. The collagen molecules then polymerize to form collagen fibrils. Lysyl oxidase is an extracellular Cu^{2+}-dependent enzyme that is also known as protein-lysine 6-oxidase. Defects in copper homeostasis, result in numerous manifestations related to defective collagen production. The final step consists of cross-link formation by the enzyme lysyl-oxidase whose two cofactors are copper and pyridoxal-phosphate; this gives the fibril the strength to perform its major tensile role. Additionally, have been examined exhaustively the incorporation of all 20 common amino acids in the X and Y positions to observe its contribution to triple-helix stability. Notably arginine in the Y position confers triple-helix stability similar to HyP (50).

There are several classes of collagenous structures; here only focus on fibrils composed primarily of type I collagen**.** Monomers of the type I collagen have the unique property of actually being unstable at body temperature. Collagen fibrils formed mainly from type I collagen (all fibrous tissues except cartilage) and fibrils formed larger from type II collagen (cartilage) have slightly different structures . Elastin is a protein that is found mainly in the ligament and walls of the blood vessels. It was isolated for the first time from aortas with copper deficiency. Elastin has low content of polar side chains especially of basic amino acids. It has a high content of glycine, proline, low hydroxyproline but does not contain hydroxylisine. Elastin contains less hydroxyproline than collagen, its content in glycine is approximately the same but does not contain the Gly-X-Y units that are repeated in the collagen.

Alteration of fibril structure .

Collagen sequence data show that in the more polar regions of the alpha chain, the tripeptide Gly-X-Y is repeated. The glycine residues are located in the center of triple helix. Each hydrogen of the –NH group of the glycine is attached to a carboxyl group of an amino acid of the neighboring chain. The peptidyl-lysyl-oxidase enzyme transforms the ε-amino group of lysine into aldehyde group. This enzyme contains copper and pyridoxal phosphate and when is inhibited by nitrile groups, then synthesis of collagen is disturbed. The lysyl-oxidase enzyme is inhibited by β-amino-propionitrilo (BAPN) a non-protein amino acid present in leguminous plant (**lathyrus sativus)**, this blocks the activity of the enzyme by kidnapping copper; in several studies it has been observed that the latirogenic compounds act only on lysyl-oxidase. The tensile effects decreased due to the fact that interlinked links are diminished. The effect of treatment with latirogen compounds causes inflammation of the cartilage and its water content increases from 20 to 30%. Collagen is distributed in all the organs of the human body and the skin, due to this fact there are injuries throughout the body during the illness.

Although genetic factors may create a predisposition towards SLE, the initiation of the disease results from several environmental triggers and exogenous factors. SLE can be induced in apparently normal subjects that are fed leguminous which contain non-protein amino acids. The toxic mechanism of non-protein amino acids is that they function as a mimics of 20 protein amino acids and are mistakenly incorporated in protein in the place of the corresponding protein amino acids similar in structure, thereby leading to the production of unnatured proteins that can not function properly (51). The toxic potential of these amino acids depend on the amount ingested and the time of consumption; and they are implicated in triggering and disease exacerbation in lupus patients . Figure 2 summarizes the role of canavanine , sunlight and some drugs in the pathogenesis of SLE . When canavanine is metabolized by the arginase enzyme canaline is produced and the latter inhibits the metabolism of ornithine, decreasing the production of polyamines and consequently decreases cell division and growth. On the other hand protein synthesis is disturb and autoantibodies are produced generating immune complexes that are deposited in the membrane of the renal glomerulus and in the collagen that supports the different organs of the human body. Canavanine disturb the collagen synthesis ; others amino acids inhibit the activity of several enzymes and others such as BAPN found in sweet bean (**lathyrus odoratus)** , specifically inhibits the formation of cross-linking of collagen and elastin chains. This legume is added to rice and consumed frequently; furthermore there are others such as alfalfa, lentil, soy bean, common bean. A major metabolite of BAPN turned out to be cyanoacetic acid. Biochemical analysis with labelled ^{14}C-BAPN shown that intramolecular cross-linking was affected. The BAPN is structurally related to the amino acid arginine. This blocks the activity of the enzyme by kidnapping copper; in several studies it has been observed that the latirogenic compounds act only on lysyl-oxidase. Arginine residues play an essential role in the assembly of vimentin from a soluble precursor to insoluble intermediate filaments ¨in vivo¨. Canavanine is characterized by metabolic disturbances in the synthesis of elastic components of collagen. (52.) In the seeds of various lathyrus species several toxic amino acids have been found such as homoarginine, mimosine and djenkolic acid in seeds, sprouts and all parts of the leguminous plant; also indospicina is incorporated into proteins, it is analogous of arginine(53). It is necessary to study others non- protein amino acids in addition canavanine to know if they have any role in the pathogenesis of the disease.

SUMMARY POINTS

1. The low levels of arginine found in the blood of patients with SLE is due to the fact that it is consumed in the synthesis of ANA. The low concentrations of arginine correlate with psychosis, stress and immunological alterations.
2. Decreases the activity of arginase by the accumulation of ornithine in the blood due to that ornithine is not metabolized.
3. When canavanine is metabolized, canaline is produced which forms a complex with pyridoxal phosphate and causes inhibition of the enzymes that metabolize ornithine.
4. The canaline inhibits the activity of 7 pyridoxal dependent enzymes: ODC, 5HTDC, OAT, TAT, OTC, DAO, peptidyl-lysyl-oxidase and in a less proportion AST and ALT.
5. The arginyl-tRNA synthetase enzyme incorporates canavanine during protein synthesis in the collagen and all proteins in the place of arginine
6. The production of autoantibodies is due to the alteration of the ionic charges in the newly synthesized peptides that have incorporated canavanine in the place of arginine

7,- Canavanine disturb the synthesis of RNA and DNA furthermore decreases the synthesis of proteins and polyamines; therefore decrease cell growth and division and is observed erythropenia, leukopenia and thrombocytopenia in patients with SLE

8.- Furthermore the inhibition of the enzymes decreases the synthesis of collagen (the peptides do not bind) and due to this fact small proline-glycine-hydroxyproline peptides are excreted in the urine of patients with SLE .

9.- Polyamines interact with DNA to protect it from denaturation and ionizing agents such as radiation from sunlight. For this reason, when the polyamines are diminished skin lesions appear due to sunlight and also because the degree of cross-linked collagen and elastin decreases

10.- All the aforementioned happens due to the action of canavanine and its metabolite the Canaline and probably others non-protein amino acids

Table 1. Prevalence of serological features in a series of at less 100 SLE patients

Serological features	prevalence (%)
Canavanine	98
Antinuclear antibodies	93
Anti-DNA antibodies	78
Arginine (decreased)	77
Antiplatelet antibodies	75
Arginase (decreased)	72
Anti-Histone antibodies	70
LE cells	70
Creatine (increased)	60
Ornithine (increased)	52
Rheumatoid factor	30
Anti-Ro (SSA) antibodies	25
Anti-Sm antibodies	20
VDRL	20

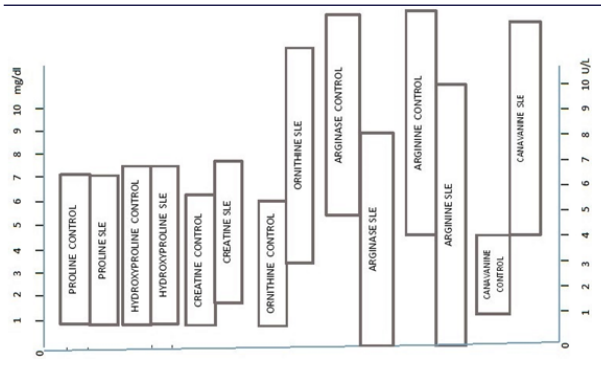

Figure 1. Comparison of arginase and amino acids values in blood samples from patients with SLE and in healthy subjects (control).

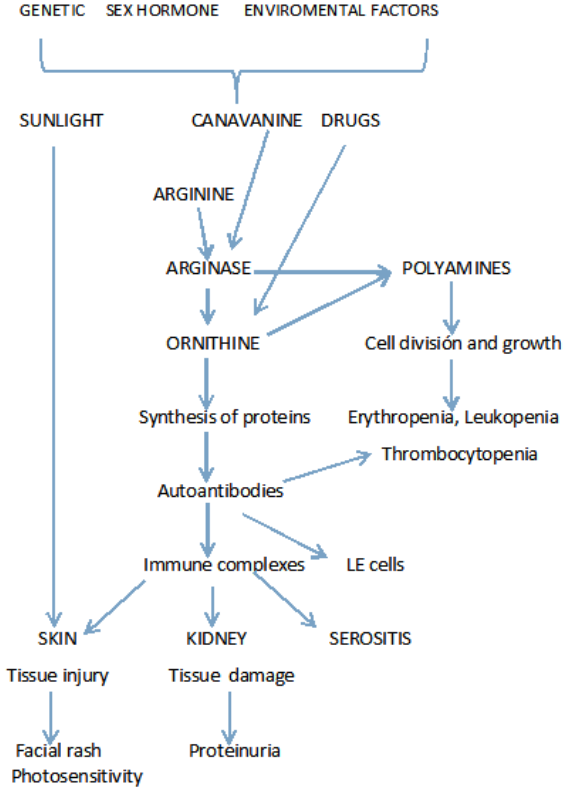

Figure 2. The pathogenesis of systemic lupus erythematosus

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