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ARGINASE LEVELS ARE INCREASED IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Arginase and nitric oxide synthase (NOS) compete for the same substrate, L-arginine. The reciprocal regulation of arginase and NOS in L-arginine-metabolizing pathways has recently been demonstrated. Since NOS is involved in the inflammation of human arthritides, we hypothesized that this reciprocal regulation might also occur within the inflamed synovium. The present study shows that both serum arginase activity and protein levels were significantly higher in patients with rheumatoid arthritis (RA) than in patients with systemic lupus erythematosus (SLE) or osteoarthritis (OA) or in healthy controls. Arginase protein concentrations in supernatants of monocyte cultures from RA patients were also significantly higher than in those from SLE or OA patients or healthy controls. In RA patients, there was a significant correlation between the serum concentrations of arginase protein and rheumatoid factor ($r=0.82$, $p<0.0001$). These data indicate that increased arginase production is seen in RA patients, but not in other immune-related diseases, suggesting that increased arginase production is unique to, and may play an important role in, the pathogenesis of RA disease.

Key words: arginase, rheumatoid factor, rheumatoid arthritis, monoclonal antibody

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Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown etiology. The inflamed synovium in RA is characterized by marked hyperplasia of the synovial lining cells, neovascularization, and massive infiltration of leukocytes[1]. Two predominant cell types, type A and type B synovial lining cells, can be recognized in the synovial lining layer[2]. Type A synoviocytes are macrophage-like cells, derived from the bone marrow, that express non-specific esterase activity, CD14, CD68, and type I complement receptors[3-5], while type B synoviocytes are fibroblast-like cells. In RA, hyperplasia of the synovial lining layer results from increased proliferation of type B synoviocytes associated with increased recruitment of type A synoviocytes from the bone marrow. In addition to marked changes in the cell lining layer of the synovial tissue, the synovial stroma is also changed. Vascular proliferation is accompanied by a massive influx of leukocytes. Leukocyte infiltration into RA synovial tissue occurs in a perivascular pattern[6]. Most of the infiltrating cells are helper T lymphocytes, accompanied by a small, but significant, number of CD8+ T cells, B cells, and macrophages[7]. A large proportion of infiltrating B lymphocytes in the RA synovium secrete rheumatoid factor (RF) [6].

Two major pathways of L-arginine metabolism are found in lymphocytes. One, involving nitric oxide synthase (NOS), results in the production of nitric oxide (NO) and citrulline, while the other, involving arginase, produces urea and L-ornithine[8-11]. Arginase exists as at least two isoforms; type I arginase (arginase I), a cytosolic enzyme, is highly expressed in the liver as a component of the urea cycle, whereas

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type II arginase (arginase II) is a mitochondrial enzyme that is expressed, to varying degrees, in many cell types [12-14]. Arginase I, originally considered exclusive to the liver, is now known to exist in polymorphonuclear leukocytes and macrophages[15,16]. The co-induction of NOS and arginase I and II activities in RAW 264.7 cells, rat peritoneal macrophages, and rat tissues by lipopolysaccharide (LPS) has been reported[10]. Since NOS and arginase compete for the same substrate, L-arginine, possible reciprocal regulation of these two pathways has recently been explored using murine macrophage cultures[10,17]. *In vivo*, this regulatory mechanism of L-arginine metabolism has been found within the glomerulus[8,18]. Since NOS is involved in the inflammation of human arthritides[19-21], we hypothesized that arginase, by reducing the amount of L-arginine, might also be involved in regulating NO production in this inflammation process in human arthritides. In this study, using a highly sensitive and specific sandwich ELISA assay for arginase protein[22] and an assay for arginase activity [23], we have shown that arginase production is increased in RA patients, but not in patients with systemic lupus erythematosus (SLE) or osteoarthritis (OA).

MATERIALS AND METHODS

Subjects

Serum samples were obtained from 25 patients with seropositive RA fulfilling the 1987 American College of Rheumatology (ACR) criteria for RA[24]. These were consecutive patients attending an outpatient clinic at the Department of Internal Medicine of the Kaohsiung Medical University Hospital. At the time of study, all RA patients were receiving non-steroid anti-inflammatory drugs, disease-modifying anti-rheumatic drugs, or less than 7.5 mg of prednisolone daily. Thirty-two patients with SLE, fulfilling the 1982 ACR criteria for SLE[25], and 12 patients with primary OA, fulfilling the 1982 ACR criteria for OA, were also enrolled to compare differences between the diseases. Fifty-eight healthy volunteers were also enrolled as normal controls. All SLE patients were receiving less than 10 mg of prednisolone daily and all OA patients were taking non-steroid anti-inflammatory drugs.

Rheumatoid factor assay

Rheumatoid factor was measured using a Beckman rate nephelometer (Beckman, Fullerton, CA) according to the manufacturer's instructions. The method employed in the Beckman RHF Test (Beckman, Fullerton, CA) measures the rate of increase in light scattering by particles formed during an antigen-antibody reaction.

Isolation and culture of peripheral blood monocytes

Twenty-five milliliters of heparinized whole blood was layered on top of 25 ml of Ficoll-Hypaque (Pharmacia-LKB, Uppsala, Sweden) and centrifuged at 400 xg for 30 min at 4℃. The plasma layer was aspirated, and the opaque interface, containing the peripheral blood mononuclear cell fraction, was harvested and washed twice with Hanks' balanced salt solution (Gibco BRL Laboratories, Grand Island, NY) containing 0.1% fetal bovine serum (Gibco BRL Laboratories, Grand Island, NY). The cells were counted, plated in a 48-well tissue culture dish at 5 x 10^6 cells/ml in culture medium, and incubated for 1 h at 37°C with agitation. Following incubation, non-adherent cells were removed, and the adherent cells (>95% pure monocytes) were cultured in culture medium for 48 h. The cell concentration was then adjusted to 2 x 10^6 cells per ml in RPMI medium (Gibco BRL Laboratories, Grand Island, NY) containing 10% fetal bovine serum. Triplicate samples (200 $\mu\ell$) of peripheral blood monocytes were placed in the wells of a microtitre plate and incubated at 37°C in 5% CO2 / 95% air for 48h, then the plates were centrifuged at 300xg at 4°C for 10 min, and the cell-free supernatants collected and stored at -80° C until used for arginase determination.

Measurement of arginase activity

Arginase activity was assayed using the method of Coulombe and Favreau[23]. Twenty microlitres of serum or macrophage cultured supernatant was incubated at 37°C for 30 min with 80 $\mu\ell$ of 125 mM L-arginine (Sigma, St. Louis, MO) in 0.1 M glycine-NaOH buffer, pH 9.5, containing 10 mM manganese chloride. The urea content of the solution was then determined by measuring the optimal density (OD) at 450 nm using a microtitre plate reader (Molecular Devices,USA).

Measurement of arginase protein

Aliquots of serum or culture supernatants of peripheral blood monocytes were assayed for arginase protein using an ELISA method[22]. Briefly, a mouse monoclonal antibody against human liver arginase (anti-arginase I mAb), produced by our laboratory[22], was used to coat the wells of flat-bottomed polyvinyl chloride microtitre plates. Triplicate samples (100 $\mu\ell$) of a 1:10 dilution of plasma or cell supernatant in phosphate-buffered saline(PBS), pH 7.4 , were then added to the plates, which were incubated for 3 h at room temperature. After washes with PBS containing 0.05%

Tween 20, horseradish peroxidase-conjugated polyclonal rabbit anti-arginase antibody was added to the plates, which were then incubated overnight at 4°C. Freshly prepared substrate-chromogen (100 $\mu\ell$ of a solution of 4 mg/ml of o-phenyldiamine in 0.1 M phosphate buffer, pH 6.0, and 0.0015% H_2O_2) was then added and incubated in the dark at 37 °C for 20 min, then the reaction was stopped by addition of 50 $\mu\ell$ of 4 M H_2SO_4 and the optical density in each well read at 490 nm (chromogen) and 650 nm (reference) in an ELISA reader. Various concentrations of arginase (0.1, 0.3, 1, 3, 10, 30, 100, and 300 ng/ml) were used to generate a standard curve from which the concentrations in the samples were calculated.

Statistics

All data are expressed as the means ± SD. Differences between groups were assessed by analysis of variance and the Scheffe test. Correlations between parameters were determined by Spearman rank order correlation. A value of $p<0.05$ was considered statistically significant. Statistical analysis were carried out using SigmaStat software (Jandel Scientific Software, San Rafael, CA, USA).

RESULTS

Serum arginase activity

Serum arginase activity was evaluated in 25 patients with RA, 32 patients with SLE, 12 patients with OA, and 58 healthy controls. As shown in Fig. 1, arginase activity in RA patients (0.508±0.166 OD) was significantly higher than that in SLE patients (0.339±0.115 OD), OA patients (0.347±0.151 OD), or normal controls (0.312±0.116 OD) ($p<0.0001$, $p=0.0079$, and $p<0.0001$, respectively). There was no significant difference between SLE patients, OA patients, and normal controls.

Serum arginase protein levels

As shown in the ELISA analysis in Fig 2, arginase protein levels in RA patients (63.9±17.8 ng/ml) were higher than those in SLE patients (26.3±14.7 ng/ml), OA patients (36.2±16.9 ng/ml), or normal controls (31.8±14.7 ng/ml) ($p<0.0001$, $p<0.0001$, and $p<0.0001$, respectively).

Arginase protein levels in monocyte culture supernatants

To measure arginase production by monocytes, adherent cells (2 x 10^6 cells/plate) were cultured for 48h and the culture supernatants harvested for analysis of arginase protein levels by ELISA. Fig 3 shows that arginase concentrations in culture supernatants from RA patients (35.3±12.9 ng/ml) were significantly higher than in those from SLE patients (14.8±5.91 ng/ml), OA patients (23.3±4.23 ng/ml), or normal controls (13.0±6.03 ng/ml) ($p=0.0053$, $p=0.0094$, and $p=0.003$, respectively).

Correlation of serum arginase protein levels and RF levels

The Spearman correlation between serum arginase levels, determined by ELISA, and RF levels in RA patients is shown in Fig. 4. Arginase levels showed a significant positive correlation with RF levels ($r = 0.82$, $p<0.0001$).

DISCUSSION

The present study clearly shows that arginase production was increased both in the serum and in supernatants from monocyte cultures from RA patients. However, no such increases were seen in SLE or OA patients. Liver-type arginase (arginase I) and the extrahepatic type (arginase II) are immunologically distinct; the arginase detected in this study was arginase I .

NOS and arginase compete for the same substrate, L-arginine. Reciprocal regulation of NOS and arginase in L-arginine-metabolizing pathways has recently been demonstrated in the glomerulus[8,18]. Since NOS is involved in the inflammation of human arthritides in which NO is produced primarily by CD14+ type A synoviocytes (derived from monocytes), endothelial cells, and chondrocytes[19-21], we hypothesized that this reciprocal regulation of L-arginine pathways might also occur within the inflamed synovium, and that arginase-producing circulating monocytes would migrate from the vascular space into the synovial stroma to compete for L-arginine with NOS, which is expressed locally in the inflammatory synovium in response to most stimuli[19-21], thus preventing sustained overproduction of NO which is toxic to surrounding cells[26]. The role of arginase in inflammation may not simply be to regulate the availability of L-arginine for enzymes, such as NOS, but also to regulate other pathways by producing ornithine[14]. Ornithine is a precursor used in the synthesis of polyamines[14] and increased generation of polyamines and high activity of their synthesizing enzyme, ornithine decarboxylase (ODC), are associated with proliferation in many cell types[27], such as activation of T lymphocytes[28] and macrophages[29], the main cells found in the rheumatoid synovium. Ornithine is also a precursor for the production of proline, which may be a rate-limiting factor in collagen synthesis

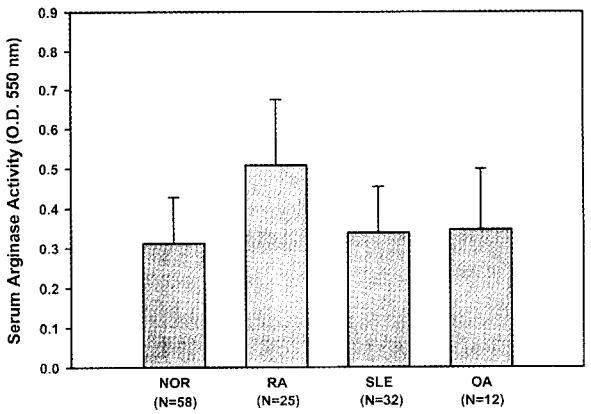

Fig. 1. Serum arginase activity in patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), or osteoarthritis (OA), or in normal controls (NOR). Values in RA patients were higher than those in SLE and OA patients and normal controls ($p<0.0001$, $p=0.0079$, $p<0.0001$, respectively).

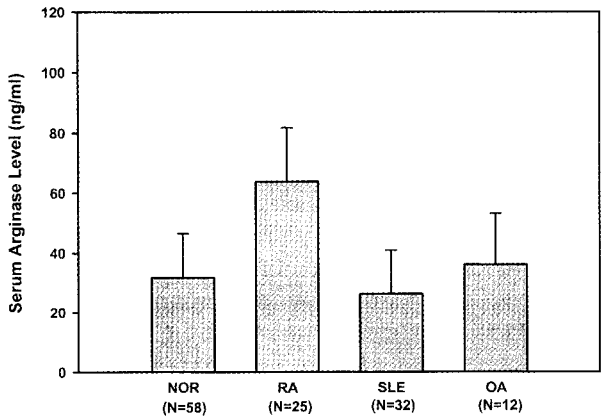

Fig. 2. ELISA measurements of serum arginase protein levels in patients and normal controls. Levels in RA patients were significantly higher than those in SLE or osteoarthritis OA patients or normal controls (NOR) ($p<0.0001$, $p<0.0001$, and $p<0.0001$, respectively).

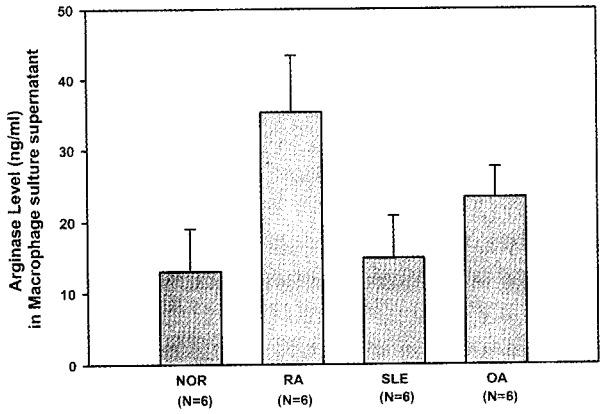

Fig. 3. Spontaneous release of arginase from cultured monocytes measured by ELISA in patients with RA, SLE, or OA, or in normal controls (NOR). The arginase protein concentration in the cell-free culture supernatant was significantly higher in RA patients than in SLE or OA patients or in NOR ($p=0.0053$, $p=0.0094$, and $p=0.003$, respectively).

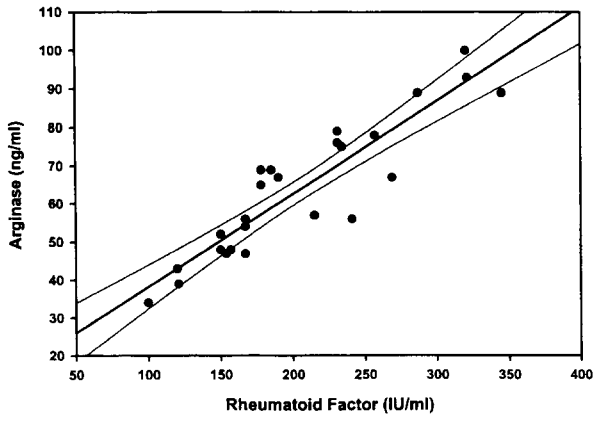

Fig. 4. Spearman correlation of serum concentrations of arginase protein and rheumatoid factor in RA patients ($n=25$, $r=0.82$, $p<0.0001$).

[30].

In the present study, arginase protein levels showed a positive correlation with RF levels. The following evidence suggests that this link between arginase and RF may be related to macrophages activated by ordinary activating factors. Firstly, arginase can be produced by monocytes from RA patients. Secondly, CD14+ monocyte-lineage cells generated from bone marrow precursors of RA patients preferentially stimulate the formation of RF by normal B cells[31]. Finally, the interaction between B cells and RA synovial macrophages inhibits B cell apoptosis and preferentially supports terminal differentiation of B cells to plasma cells, thus favoring high levels of RF production in the rheumatoid synovium [32, 33].

Our data show that arginase production is increased in RA, but not in other immune-related diseases, suggesting that arginase activity may play an important role in the pathogenesis of RA disease. In addition, detection of serum arginase levels may be of use in the diagnosis and prognosis of RA disease.

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REFERENCES

1. Ziff M. Pathways of mononuclear cell infiltration in rheumatoid synovitis. Rheumatol Int 1989;9: 97-103.
2. Barland P, Novikoff AB, Hamerman D. Electron microscopy of the human synovial membrane. J Cell Biol 1962;14:207-220.
3. Wilkinson LS, Pitsillides AA, Worrall JG, Edwards JC. Light microscopic characterization of the fibroblast-like synovial intimal cell (synoviocyte). Arthritis Rheum 1992;35: 1179-1184.
4. Edward JC, Willoughby DA. Demonstration of bone marrow derived cells in synovial lining by means of giant intracellular granules as genetic markers. Ann Rheum Dis 1982; 41: 177-182.
5. Edward JC. The origin of type A synovial lining cells. Immunobiology 1982;161: 227-231.
6. Liao HX, Haynes BF. Role of adhesion molecules in the pathogenesis of rheumatoid arthritis. Rheum Dis Clin North Am 1995; 21: 715-739.
7. Kohem CL, Brezinschek RI, Wisbey H, Tortorella C, Lipsky PE, Oppenheimer-Marks N. Enrichment of differentiated CD45RBdim, CD27-memory T cells in the peripheral blood, synovial fluid, and synovial tissue of patients with rheumatoid arthritis. Arthritis Rheum 1996; 39: 844-854.
8. Cook HT, Jansen A, Lewis S, Largen P, O'Donnell M, Reaveley D, Cattell V. Arginine metabolism in experimental glomerulonephritis : interaction between nitric oxide synthase and arginase. Am J Physiol 1994; 267: F646-653.
9. Hrabak A, Idei M, Temesi A. Arginine supply for nitric oxide synthesis and arginase is mainly exogenous in elicited murine and rat macrophages. Life Sci 1994; 55:797-805.
10. Wang WW, Jenkinson CP, Griscavage JM, Kern RM, Arabolos NS, Byrns RE, Cederbaum SD, Ignarro LJ. Co-induction of arginase and nitric oxide synthase in murine macrophages activated by lipopolysaccharide. Biochem Biophys Res Commun 1995; 210:1009-1016.
11. Chang CI, Liao JC, Kuo L. Arginase modulates nitric oxide production in activated macrophages. Am J Physiol 1998; 274 :H342-348.
12. Gotoh T, Araki M, Mori M. Chromosomal localization of the human arginase II gene and tissue distribution of its mRNA. Biochem Biophys Res Commun 1997; 233:487-491.
13. Morris SM Jr, Bhamidipati D, Kepka-Lenhart D. Human type II arginase : sequence analysis and tissue-specific expression. Gene 1997;157-161.
14. Morris SM Jr, Kepka-Lenhart D, Chen LC. Differential regulation of arginase and inducible nitric oxide synthase in murine macrophage cells. Am J Physiol 1998; 275:E740-747.
15. Klein D, Shapiro BJ, Roberts R, Grody WW, Kern R, Ganz T, Stiehm FR, Cederbaum SD. Differential expression of human arginase in cells of bone marrow origin. Am J Human Genetics 1998; 43:A-131.
16. Sonoki T, Nagasaki A, Gotoh T, Takiguchi M, Takeya M, Matsuzaki H, Mori M. Coinduction of nitric oxide synthase and arginase I in cultured rat peritoneal macrophages and rat tissues in vivo by lipopolysaccharide. J Biol Chem 1997; 272: 3689-3693.
17. Modolell M, Corraliza IM, Link F, Soler G, Eichmann K. Reciprocal regulation of the nitric oxide synthase/ arginase balance in mouse bone marrow-derived macrophages by Th1 and Th2 cytokines. Eur J Immunol 1995; 25: 1101-1104.
18. Jensen A, Lewis S, Cattell V, Cook HT. Arginase is a major pathway of L-arginine metabolism in nephritic glomeruli. Kidney Int 1992; 42:1107-1112.
19. Sakurai H, Kohsaka H, Liu MF, Higashiyama H, Hirata Y, Kanno K, Saito I, Miyasaka N. Nitric oxide production and inducible nitric oxide synthase expression in inflammatory arthritides. J Clin Invest 1995;96:2357-2367.
20. Ueki Y, Miyake S, Tominaga Y, Eguchi K. Increased nitric oxide levels in patients with rheumatoid arthritis. J Rheumatol 1996; 23:230-236.
21. Farrell AJ, Blake DR, Palmer RMJ, Moncada S. Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic disease. Ann Rheum Dis 1992; 151: 1219-1222.
22. Huang LW, Liu HW, Chang KL. Development of a sandwich ELISA test for arginase measurement based on monoclonal antibodies. Hybridoma 2001; 20:53-57.
23. Coulombe JJ, Favreau LA. A new simple semi-micro method for colorimetric determination of urea. Clin Chem 1963; 9:102-108.
24. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31:315-324.
25. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis

Rheum 1982;25:1271-1277.
26. Gotoh T, Mori M. Arginase II downregulates nitric oxide (NO) production and prevents NO-mediated apoptosis in murine macrophages derived RAW 264. 7 cells. J Cell Biol 1999; 144:427-434.
27. Tabor CW, Tabor H. Polyamines. Ann Rev Biochem 1984; 53 : 749- 790.
28. White MW, Kameji T, Pegg AE, Morris DR. Increased efficiency of translation of ornithine decarboxylase mRNA in nitrogen-activated lymphocytes. Eur J Biochem 1987; 170:87-92.
29. Messina L, Arcidiacono A, Spampinato G, Malaguarnera L, Berton G, Kaczmarek L, Messina A. Accumulation of ornithine decarboxylase mRNA accompanies activation of human and mouse monocytes/macrophages. FEBS Letters 1990;268:32-34.
30. Rojkind M, Deleon D. Collagen biosynthesis in cirrhotic rat liver slices: a regulatory mechanism. Biochim Biophys Acta 1970; 217:512-522.
31. Hirohata S, Yanagida T, Koda M, Koiwa M, Yoshino S, Ochi T. Selective induction of IgM rheumatoid factors by CD14+ monocyte-lineage cells generated from bone marrow of patients with rheumatoid arthritis. Arthritis Rheum 1995; 38:384-388.
32. Dechanet J, Merville P, Durand I, Banchereau J, Miossec P. The ability of synoviocytes to support terminal differentiation of activation B cells may explain plasma cell accumulation in rheumatoid synovium. J Clin Invest 1995; 95:456- 463.
33. Merville P, Dechanet J, Desmouliere A, Durand I, de Bouteiller O, Garrone P, Banchereau J, Liu YJ. Bcl-2+ tonsillar plasma cells are rescued from apoptosis by bone marrow fibroblasts. J Exp Med 1996; 183:227-236.

類風濕性關節炎患者血清中具有高濃度精胺酸酶

黃莉文 張基隆 * 陳忠仁 ** 劉宏文 **

精胺酸酶和一氧化氮合成酶會競爭相同的受質 L- 精胺酸，在精胺酸代謝途徑中，有學者研究指出精胺酸酶和一氧化氮合成酶彼此之間的具互相調節功用。一氧化氮合成相酶與人類關節疾病之發炎反應相關，據此推測在發炎性關節滑膜組織內也可能存在著這兩種酵素互相調節。我們研究結果顯示：類風濕性關節炎病人血清精胺酸酶活性及濃度均明顯高於紅斑性狼倉病人，骨關節炎病人及正常人。在類風濕性關節炎病人單核球培養上清液所測得之精胺酸酶濃度也明顯高於紅斑性狼瘡病人，骨關節炎病人及正常人。類風濕性關節炎病人血清精胺酸酶濃度與其類風濕因子之間出現明顯正相關($r = 0.82$, $p<0.0001$)。這些資料顯示類風濕性關節炎病人可見精胺酸酶生成量增加，其它免疫疾病則否。此顯示出精胺酸酶生成量之增加是類風濕性關節炎所特有的現象之一，且在類風濕性關節炎致病機轉上扮演重要角色。

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