# AMINO ACID COMPOSITION OF LYMPH AND BLOOD IN ALLERGY

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Views on disturbances of protein metabolism in allergy are mainly based on electrophoretic investigation of the protein composition of the peripheral blood. Although the general trend of changes in protein metabolism during allergic reactions has been elucidated, many questions on the redistribution of plasma proteins and of their low-molecular-weight structural components (amino acids) in the blood and lymph still remain unanswered. The important role of the lymphatic system in regulation of the intensity of metabolic processes in the intercellular interstitial spaces of tissues and organs (resorption, transport, hemostatic, and other functions of the lymphatic system) likewise must be mentioned.

The aim of this investigation was to compare the time course of free amino acid and total protein levels in the lymph and peripheral blood of dogs during sensitization and anaphylactic shock (AS).

## EXPERIMENTAL METHOD

Experiments were carried out on 23 mongrel dogs of both sexes weighing from 4 to 20 kg. The animals were sensitized by triple subcutaneous injection of normal horse serum (NHS) in a dose of 6.4 mg/kg. On the 21st-23rd days after the last sensitizing injection the animals were anesthetized for use in acute experiments with 3% thiopental (20 mg/kg) and the reacting dose of antigen (32 mg/kg) was injected. Lymph was withdrawn through a cannula from the left thoracic lymphatic duct where it drains into the mouth of the jugular vein. Throughout the experiment the blood pressure and respiration of the experimental animals were recorded on a kymograph. The total protein concentration in the venous blood plasma and lymph was determined with an IRF-22 refractometer, and free amino acid levels were determined with an AAA-8881 automatic amino-acid analyzer before injection of the reacting dose of antigen and also 30 and 60 min after the beginning of AS. The animals were killed by injection of a lethal dose of the anesthetic. The experimental results were subjected to statistical analysis [7].

## EXPERIMENTAL RESULTS

It will be clear from Tables 1 and 2 that under normal conditions blood levels of amino acids (except glutamic acid, methionine, and lysine) are significantly higher than their levels in the lymph. During sensitization the concentrations of free amino acids in the lymph were increased. As a rule their levels in the lymph (except for cysteine) were several times higher: 2-3 times for glutamic acid, isoleucine, leucine, and methionine, 4-5 times for alanine, glycine, threonine, serine, valine, and lysine, and actually more than 10 times for aspartic acid. Unlike changes in amino acid levels in the lymph, in the blood plasma an increase was observed only in the concentrations of glycine, lysine, and aspartic acid.

AS was accompanied by a sharp increase in the plasma concentrations of all amino acids. In the first 30 min of shock, levels of serine, cysteine, lysine, and aspartic and glutamic acids rose by more than 3-3.5 times, of isoleucine by 7.5 times, and of methionine by 10 times.

A significant increase in the concentrations of threonine, methionine, and cysteine in the lymph was observed in AS. A marked tendency also was observed for the concentrations of glycine, valine, and leucine in the lymph to be increased 30 min after injection of NHS, whereas concentrations of aspartic and glutamic acids and of serine and threonine were reduced

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TABLE 1. Concentrations of Total Protein (in g/liter) and Free Amino Acids (in μmoles/liter) in Thoracic Duct Lymph of Dogs under Normal Conditions, at Height of Sensitization, and in Course of AS (n = 15)

| Amino acid | Normal (n = 10) | Before injection of reacting dose of antigen | After injection of reacting dose of antigen | |
|---|---|---|---|---|
| | | | 30 min | 60 min |
| Aspartic | 2,479±0,451 | 27,419±16,526† | 25,015±18,254 | 9,991±16,752† |
| Glutamic | 39,01±6,797 | 84,959±9,651** | 81,017±8,564 | 40,508±9,787* |
| Alanine | 20,539±7,856 | 113,692±14,141‡ | 119,529±20,539 | 95,398±23,906 |
| Glycine | 17,051±2,131 | 78,060±6,660* | 109,231±2,011 | 93,513±23,844 |
| Serine | 13,321±0,381 | 48,811±4,853* | 68,030±9,039 | 40,723±4,472 |
| Cysteine | 9,987±6,520 | 10,730±3,466 | 53,070±5,777‡ | 53,566±8,996‡ |
| Valine | 15,499±1,707 | 71,868±22,704† | 101,656±29,959 | 77,330±38,750 |
| Isoleucine | 5,489±0,839 | 16,695±1,601‡ | 17,838±2,973 | 23,174±9,529 |
| Leucine | 12,573±1,677 | 36,286±4,650‡ | 54,581±9,376 | 51,761±14,713 |
| Threonine | 11,501±1,427 | 56,246±9,402‡ | 62,374±2,099† | 37,693±9,066 |
| Methionine | 2,614±0,469 | 6,634±1,407† | 10,253±0,804‡ | 7,305±1,005 |
| Lysine | 17,580±6,430 | 71,688±7,661‡ | 61,222±8,140 | 62,863±18,538 |
| | | | 5—8 min / 30 min | 60 min / 120 min |
| Total protein | 32,8±0,9 | 34,3±2,4 | 36,8±1,9 / 10,3±2,3** | 41,8±2,8**** / 43,7±2,5* |

Legend. *P < 0.001, †P < 0.05, ‡P < 0.01, **P < 0.02.

TABLE 2. Concentrations of Total Protein (in g/liter) and Free Amino Acids (in μmoles/liter) in Blood Serum of Dogs under Normal Conditions, at Height of Sensitization, and in Course of AS (n = 15)

| Amino acid | Normal (n = 10) | Before injection of reacting dose of antigen | After injection of reacting dose of antigen | |
|---|---|---|---|---|
| | | | 30 min | 60 min |
| Aspartic | 10,892±3,005 | 32,903±7,061† | 101,863±9,991* | 84,360±8,338* |
| Glutamic | 36,430±5,369 | 38,062±9,447 | 152,790±15,496† | 129,002±16,244* |
| Alanine | 104,153±19,416 | 72,503±9,764 | 168,575±30,527† | 150,056±21,212‡ |
| Glycine | 61,276±13,332 | 84,055±15,452† | 131,078±15,053†** | 96,976±10,124 |
| Serine | 27,402±11,893 | 39,772±6,755 | 112,845±5,043‡ | 92,674±15,128† |
| Cysteine | 12,545±4,044 | 6,355±1,155 | 20,551±3,800‡ | 18,488±6,355 |
| Valine | 54,114±7,596 | 53,260±6,828 | 125,555±16,473‡ | 96,023±18,010 |
| Isoleucine | 8,538±1,678 | 12,883±1,753 | 93,002±10,215** | 67,998±5,107** |
| Leucine | 20,277±2,592 | 26,910±6,632 | 181,278±6,376* | 156,655±12,654** |
| Threonine | 42,478±0,402 | 44,493±4,197 | 94,694±10,326* | 75,134±10,410† |
| Methionine | 2,949±1,541 | 4,088±1,474 | 44,498±11,259‡ | 35,451±8,310‡ |
| Lysine | 9,850±3,283 | 34,886±9,440† | 175,115±21,068* | 127,574±9,850** |
| | | | 5—8 min / 30 min | 60 min / 120 min |
| Total protein | 68,0±1,2 | 66,1±1,5 | 64,7±0,5‡ / 64,3±1,2† | 64,2±0,9† / 62,8±1,2‡ |

Legend. *P < 0.01, †P < 0.05, ‡P < 0.02, **P < 0.001.

1 h after the beginning of shock (compared with their level before injection of the reacting dose of the antigen). The results of investigation of the amino-acids spectrum in the lymph in AS showed no relationship between structure and changes in their level: Changes in the same direction were found with amino acids belonging to different classes, and changes in opposite directions with amino acids belonging to the same class.

Antigenic stimulation was accompanied by a tendency for the total protein concentration in the lymph to rise, and a distinguishing feature of changes in the protein composition of the lymph in the course of AS was that its concentration remained high, reaching maximal values 2 h after the beginning of shock (whereas the total protein concentration in the blood serum fell).

An essential role in the quantitative and qualitative changes in the amino-acid composition of the body fluids during sensitization is evidently played by the "proteolytic background" resulting from labilization of lysosomal membranes [1, 14]. We also know that sensitization is accompanied by marked activation of the glucocorticoid function of the adrenal cortex [2, 4, 8], which mobilizes amino acids from the tissues, especially lymphoid tissue, and stimulates their utilization in the liver for gluconeogenesis and protein synthesis in the hepatocytes [5, 6, 9, 10, 12]. The significant increase in concentration of amino acids in the lymph compared with that in the blood plasma is also evidence of their lymphoid origin.

We also know that continuous protein synthesis from amino acids takes place in the body cells and that the newly formed protein either is utilized by the cells of the particular tissue concerned or enters the circulation via the lymphatic system. It can be tentatively suggested that as a result of the increase in vascular permeability accompanying allergic sensitization the protein concentration in the interstitial tissue spaces (shown by elevation of the total protein level in the lymph in the present experiments) reaches a "critical value," with the result that a response of feedback type occurs: An increase in the protein concentration in the interstitial spaces causes inhibition of protein synthesis. Free amino acids thereupon are resorbed by the lymphatic capillaries.

As regards the biological significance of the imbalance in the amino-acid concentration in the lymph and blood developing during sensitization, the following comments may be made. During active transmembrane transport of amino acids, coupled with energy consumption, complex relations (competitive and synergic) are established between them. Similar reactions between amino acids also exist in the course of protein biosynthesis: mutual activation in some, mutual inhibition of their incorporation into protein in others. A deficiency of some amino acids and a relative excess of others in the amino acid pool affect the quality of the synthesized proteins. It may be assumed that an excess of one particular amino acid may inhibit incorporation of some amino acids and, at the same time, stimulate incorporation of others. All this may lead to a serious change in the primary structure of proteins and to the appearance of biologically inactive proteins, instead of biologically active, with consequent deprivation of subcellular structures of their physiologically essential functions, and with disturbances of function and structure in the tissues and organs [3, 11, 12].

The sharp rise in the plasma amino-acid level in AS can be associated with activation of proteolysis, under the influence of the antigen–antibody reaction, actually in the general circulation (esterases of the first component of complement, plasminogen–plasmin and peptidase systems, liberation of lysosomal enzymes from leukocytes, platelets, and also endothelial cells as a result of their allergic alteration, etc.). Evidence of this is given by the smaller changes in the free amino acid level in the lymph. During AS, as a result of allergic injury to the endothelial cells of the vascular wall (in particular, a reduction in their energy supply), circulatory disturbances, and other changes, the foundations are laid for a disturbance of the transendothelial amino acid transport system, and this must evidently lead to some degree of amino acid accumulation in the general circulation. Similar disturbances of amino acid transport probably take place also at the level of endothelial cells of lymphatic terminals.

The marked increase in total protein concentration in the lymph and its decrease in the blood during AS are evidence of a generalized increase in vascular permeability under the influence of vasoactive substances (mediators of immediate allergy): an increase in area of functioning capillaries, activation of hyaluronidase, depolymerization of mucopolysaccharides of the ground substance of connective tissue and a decrease in its viscosity, an increase in size of the interendothelial spaces, intensification of transendothelial transport of liquid and solid substances and, as a result of this, an increase in vascular permeability, in lymph formation, and an increase in the protein concentration in the lymph all take place. The increase in the protein concentration in the lymph is evidently due mainly to an increase in capillary permeability in the splanchnic system and in the liver. The volume and composition of lymph in the thoracic duct, under natural conditions, is known to be largely determined by the state of function of the abdominal organs; quantitative changes in the lymph flow mainly depend on intestinal activity, biochemical changes mainly on liver function. An increase in capillary permeability and congestive disturbances in the hepatoportal system in AS undoubtedly lead to an increase in the total protein concentration in lymph of the thoracic duct.

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# SPONTANEOUS AND MITOGEN-INDUCED PROLIFERATIVE ACTIVITY OF MONONUCLEAR CELLS IN PATIENTS WITH POLLINOSIS

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The increased number of B lymphocytes [6] and changes in representation of membrane immunoglobulins in B cells depending on the stage of an allergic disease [17] suggest changes in functional activity of individual B-cell subpopulations. If it is recalled also that an increased number of "null" cells [2] is found in atopic patients and, according to some workers [15], the "null" cells include precursors of mature B lymphocytes, the need to study proliferative activity of B cells in atopic patients will be evident.

In the activation described below induction of the proliferative response of B cells by lipopolysaccharide (LPS) and the proliferative activity of T lymphocytes during stimulation by phytohemagglutinin (PHA) in patients with pollinosis, with hypersensitivity to timothy-grass pollen, were studied.

## EXPERIMENTAL METHOD

Peripheral blood mononuclear cells (MNC) from 17 patients with pollinosis aged from 16 to 40 years, were used. The diagnosis of pollinosis was based on the allergologic history and the results of skin tests and inhalation provocation tests with the possible allergen. The duration of the disease did not exceed 5-7 years. Patients with pollinosis were studied while free from seasonal exacerbations and accompanying diseases, and who had not previously been subjected to specific hyposensitization or pharmacotherapy, were chosen. Patients with a moderately severe and severe degree of clinical manifestations of pollinosis with positive skin tests to timothy grass pollen allergen were investigated. The control consisted of 13 healthy blood donors. In a separate series of investigations the paired control method was used. For this purpose clinically healthy persons were selected individually to correspond to sex, age, and race to a patient chosen for investigation, and with subsequent parallel conduct of experiments.

Lymphocytes (MNC) were isolated from heparinized blood by centrifugation on a one-step Ficoll–Verografin gradient with density of 1.080 g/cm$^3$ at 400g for 40 min, followed by washing with medium 199 at 200g three times, for 5 min each time. MNC were resuspended in medium 199 containing 10% embryonic calf serum, HEPES (5 mM), glutamine (20 mM), and monomycin (100 U/ml), and cultured in flat-bottomed microplates at the rate of $3.6 \times 10^5$ cells per well in volume of 200 µl of medium for 7 days at 37°C at an atmosphere of 5% $CO_2$.

LPS from *E. coli* serotype 026:B6 (Sigma, USA) in final concentrations of 2, 20, and 100 µg/ml, was used as B-cell activator. The available information on the use of LPS as B-cell mitogen for human peripheral blood lymphocytes is highly contradictory. In particular, during culture for 3 days with LPS the proliferative response of MNC was negligible [3] or absent

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