IMMUNOLOGY

Functional disturbances of immune response in different periods of experimental pneumonia development

O. O. Chugay

Danylo Halytsky Lviv National Medical University

Paper received 13.06.17; Accepted for publication 20.06.17.

Abstract. One of the main mechanisms of protecting immune system against pathogens is functioning of different parts of the immune system of respiratory mucosa. At the same time, there was activation of humoral immunity, manifested by increase in B-lymphocyte level and circulating immune complexes in the blood both in early and late periods of experimental pneumonia. This condition may be caused by the nature of the agent. Since experimental pneumonia in guinea pigs was provoked by *Staphylococcus aureus*, which is a gram-positive bacterium, humoral immunity plays a leading role in combating this infection.

Keywords: *experimental pneumonia, T-lymphocytes, B-lymphocytes, circulating immune complexes.*

Introduction. Despite considerable advances in combating diseases, pneumonia remains a significant medical and social problem in most countries of the world in the 21st century. According to WHO definition, pneumonia is an acute, as a rule, infectious disease, predominantly of the respiratory part of the lungs with the presence of exudate in the alveoli, which contains neutrophils and is manifested by infiltrated darkening of the lungs on X-ray [1].

A significant part of the surface of lung epithelium and massive capillary network for gas exchange make the lungs especially vulnerable to the environment. One of the basic mechanisms of protection against pathogens is immune system of the respiratory mucosa, and first of all, its cell section. For this, there is the whole network of specialized cells, which includes secretory Clara cells, ciliated, goblet and submucous glandular cells. The basic cells of myeloid origin in lung lumen are alveolar macrophages, which possess high phagocytic activity. Due to these cells, low-virulence microorganisms are effectively destroyed in the lungs and mechanisms of acquired immune response become activated [2]. Antigen-specific proliferation and differentiation of T- and B-lymphocytes are referred to the mechanisms of specific response [3].

CD3 /4+ T-lymphocyte-helpers stimulate conversion of B-lymphocytes in plasma cells, activate dendrite cells and macrophages producing cytokines and co-stimulating molecules, initiate proliferation of T-cytotoxic lymphocytes [4]. CD3/8+ T-cells produce a significant amount of pro-inflammatory cytokines and directly destroy body's own cells, which are infected with viruses or other pathogenic intracellular microorganisms, as well as atypical cells. The phenotype of T-cytotoxic lymphocytes – γ- i δ-T-cells (intraepithelial) and invariable natural killer T-cells (iNKT), similarly as innate lymphoid cells (ILCs) play a significant role in early response to lung infection [6].

B-lymphocytes provide humoral immune reaction producing antibodies. Activation of mature B-cells occurs in their contact with antigen epitopes, which are recognized by surface receptors of immunoglobulins (Ig). By means of these receptors, B-lymphocytes may recognize not only peptides, but also large molecules of proteins, nucleic acids, carbohydrates, lipids, polysaccharide and soluble lipoprotein antigens [5, 7].

Humoral response to more complex antigens, including proteins, depends on T-helpers. T-cell activation of B-lymphocytes – is a complex system of interaction between antigen-presenting cells, antigen, B- and T-lymphocytes. As a result of a cascade of reactions involving co-stimulating molecules and cytokines, B-lymphocytes are activated, they proliferate and differentiate into plasma cells, which produce specific antibodies.

One of the most important biological functions of immunoglobulins is their binding with an antigen and formation of immune complex (IC). Formation of immune complex is one of the components of physiological immune response, which permanently occurs in the human body, aimed at maintenance of internal homeostasis and is the final stage of immune protection against foreign allergens [11].

The aim of our investigation was to study peculiarities of impairment of cell-mediated and humoral immune responses under the conditions of experimental pneumonia development (EP).

Materials and methods of investigation. Experimental studies were performed on 32 guinea pigs (mammals) weighing 180-220 g, divided into 4 groups, 8 animals in each of them:

- I group – intact guinea pigs (control);
- II group – guinea pigs with experimental pneumonia on the 6th day;
- III group – guinea pigs with experimental pneumonia on the 10th day;
- IV group – guinea pigs with experimental pneumonia on the 20th day.

For rational interpretation of the obtained digital data, two periods (early and late) of EP development were differentiated. Early period involved group pf animals with EP on the 6th and 10th days of experiment, and late period – on the 20th day.

All animals were kept in standard conditions of the vivarium of Danylo Halytsky Lviv national medical university. Euthanasia was performed decapitating the animals, according to European Convention for the Protection of Vertebrate Animals, used for experimental and scientific purposes (Strasbourg, 1985), Directive of European Council 86/609/EEC (1986), Law of Ukraine № 3447-IVIV "On protection of animals from cruel treatment", approved by First national congress of Ukraine on bioeth-

ics (2001).

EP was imitated by intranasal introduction of *Staphylococcus aureus* to animals by the method of V.N. Shlyapnikov et.al. [12].

Then, the animals were decapitated on the 6th, 10th and 20th day of EP development under ether anesthesia, and blood samples were taken for immunological investigation. Amount of T- and B-lymphocytes (CD3 and CD19) in the blood was measured by the method of E.F. Chernushenko, L.S. Kohosov [13]. The level of circulating immune complexes (CIC) in the blood was measured by the method of V. Haskova, J. Kaslik [14].

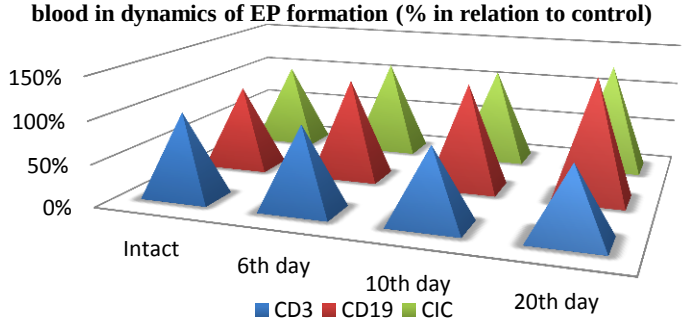
The obtained results were statistically evaluated by Student's t-criterion. The data are presented as mean value (M) based on the results of each investigation ± standard deviation (m). Reliable differences were at $p<0.05$ (95.5%).

Results of investigation and their discussion. The results of immunological investigation showed that in early period, which includes the 6th and the 10th days of EP development, significant changes of certain indices of cell-mediated and humoral immunities occur.

In particular, it was detected that amount of T-lymphocytes in the blood of guinea pigs decreased by 1.8% on the 6th day of EP, as compared with control. Further, a reliable reduction of appropriate indices by 11.74% ($p<0.05$) was observed on the 10th day of the experiment as compared with intact animals, which indicated inhibition of cell-mediated immune response.

The condition of humoral immunity was evaluated by detecting amount of B-lymphocytes and CIC. In early period of EP, particularly on the 6th day, increase in B-lymphocytes by 19.45% ($p<0.05$) was observed, as compared with control. Further elevation of B-lymphocytes in the blood by 25% ($p<0.05$) was seen on the 10th day of the experiment, as compared with intact group. Elevation of CIC level occurred on the 6th day by 13.68% ($p<0.05$) in relation to control. However, on the 10th day of experiment we did not notice further increase in CIC amount. Their level remained the same as on the 6th day, thus, it was reliably by 13.5% ($p<0.05$) higher only in relation to control group (Fig. 1).

Fig.1. Amount of T- and B-lymphocytes and CIC in guinea pigs' blood in dynamics of EP formation (% in relation to control)



Thus, the conducted analysis of investigation of certain indices of immune system on the 6th and 10th days of experiment showed elevation of B-lymphocytes and CIC and reduction of T-lymphocytes in guinea pigs' blood, which indicates impairment of immune response in early period of EP formation.

In late period of EP development, particularly on the 20th day, further reduction of T-lymphocytes by 19.6% ($p<0.05$) in relation to control group and by 7.9% ($p<0.05$), as compared with the 10th day, was recorded. At the same time, indices of humoral immunity continued to increase. In particular, the levels of B-lymphocytes and CIC increased by 43.65% and 29.8% ($p<0.05$), respectively, in relation to control group and reliably ($p<0.05$) increased in comparison with early period of EP (Fig. 1).

Thus, late period of EP was characterized by further impairment of functional activity of cell-mediated and humoral immune responses.

Conclusion. Both early and late periods of EP are characterized by impairment of immune response to invaded pathogen. Experimental pneumonia in guinea pigs was accompanied by inhibition of cell-mediated immunity, which was manifested by reduction of T-lymphocytes in the blood. At the same time, it was accompanied by activation of humoral immunity, which was manifested by an increase in the level of B-lymphocytes and CIC in the blood both in the early and late periods of EP. This condition can be provoked by the nature of the agent. Since EP in guinea pigs was caused by *Staphylococcus aureus*, which is a gram-positive bacterium, humoral immunity plays the main role in combating this infection.

ЛИТЕРАТУРА

1. Дранник Г. Л. Клиническая иммунология и аллергология / Г. Л. Дранник. – Киев: ООО «Полиграфплюс», 2010. – 552 с.
2. Chen K. T cell-mediated host immune defenses in the lung / K. Chen, J. K. Kolls. // Annu Rev Immunol. – 2013. – №31. – P. 605–33.
3. Moore B. B. Role of T- and B-lymphocytes in pulmonary host defences / B. B. Moore, T. A. Moore, G. B. Toews. // Eur Respir J. – 2001. – №18. – P. 846–56.
4. Marshall N. B. Cytotoxic CD4 T cells in antiviral immunity / N. B. Marshall, S. L. Swain. // J Biomed Biotechnol. – 2011; 2011:954602.
5. Посібник для проведення практичних занять з клінічної імунології та алергології/ [В.В. Чоп'як, Г.О.Потьомкіна, А.М. Гаврилюк та ін.] – Львів: Видавець Тетюх Т.В., 2015. – 208 с.
6. Імунологія: підручник / [Л. В. Кузнецова, В. Д. Бабаджан, Н. В. Харченко та ін.]. – Вінниця: ТОВ «Меркьюрі Поділля», 2013. – 526 с.
7. Signalling in B cells / [J. E. Ales-Martinez, E. Cuende, C. Martinez et al.]. // Immunol Today. – 1991. – №12. – P. 201–205.
8. Kuby J. Immunology. 2nd Edn. / J. Kuby. – New York: W.H. Freeman and Company, 1994.

9. Parker D. C. T cell-dependent B cell activation / D. C. Parker. // Annu Rev Immunol. – 1993. – №11. – P. 331–60.
10. Tonegawa S. Somatic generation of antibody diversity / S. Tonegawa. // Nature. – 1983. – №302. – P. 575–581.
11. Атаман О. В. 3. Патофізіологія. Т.1. Загальна патологія / О. В. Атаман. – Вінниця: Нова книга, 2012. – 592 с.
12. Экспериментальные модели острых пневмоний, вызванных условно-патологическими бактериями и их ассоциацией: метод. указания / [В. И. Шляпников, Т. Л. Солодова, С. А. Степанов и др.]. – Саратов, 1988. – 30 с.
13. Чернушенко Е. Ф. Иммунология и иммунопатология заболеваний легких / Е. Ф. Чернушенко, Л. С. Когосова. – К. : Здоров'я, 1981. – 208 с.
14. Haskova V. Novy zpusob stanoveni circulujicich imunokomplexy w lidskych serech / V. Haskova, J. Kaslik, M. Matejckava // Cas. Lek. Ces. – 1977. – T. 116, № 14. – S. 436–437.

REFERENCES

1. Drannik H.L. Clinical immunology and allergology / H.L. Drannik. – Kiev: OOO "Polygraphplus", 2010. – 552 p.
2. Chen K. T cell-mediated host immune defenses in the lung / K. Chen, J. K. Kolls. // Annu Rev Immunol. – 2013. – №31. – P. 605–33.
3. Moore B. B. Role of T- and B-lymphocytes in pulmonary host defences / B. B. Moore, T. A. Moore, G. B. Toews. // Eur Respir J. – 2001. – №18. – P. 846–56.
4. Marshall N. B. Cytotoxic CD4 T cells in antiviral immunity / N. B. Marshall, S. L. Swain. // J Biomed Biotechnol. – 2011; 2011:954602.
5. Textbook for conduction of practical classes in clinical immunology and allergology / [V.V. Chopyak, H.O. Potiomkina, A.M. Havryliuk et. al.] – Lviv: Editor Tetiuh T.V., – 208 p.
6. Immunology: textbook / [L. V. Kuznetsova, V.D. Babadzhan, N.V. Kharchenko et. al.]. – Vinnytsya: TOV "Merkiuri Podillya", 2013. – 526 p.
7. Signalling in B cells / [J. E. Ales-Martinez, E. Cuende, C. Martinez et al.]. // Immunol Today. – 1991. – №12. – P. 201–205.
8. Kuby J. Immunology. 2nd Edn. / J. Kuby. – New York: W.H. Freeman and Company, 1994.
9. Parker D. C. T cell-dependent B cell activation / D. C. Parker. // Annu Rev Immunol. – 1993. – №11. – P. 331–60.
10. Tonegawa S. Somatic generation of antibody diversity / S. Tonegawa. // Nature. – 1983. – №302. – P. 575–581.
11. Ataman O.V. Pathological physiology. V.1. General pathology / O.V. Ataman. – Vinnytsya: Nova knyha, 2012. – 592 p.
12. Experimental models of acute pneumonia, caused by conditionally pathogenic bacteria and their associations: methodical recommendations / [V. I. Shlyapnikov, T.L. Solodova, S.A. Stepanov et. al.]. – Saratov, 1988. – 30 p.
13. Chernushenko E.F. Immunology and immunopathology of lung diseases / E.F. Chernushenko. – Kyiv: Zdorovya, 1981. – 208 p.
14. Haskova V. Novy zpusob stanoveni circulujicich imunokomplexy w lidskych serech / V. Haskova, J. Kaslik, M. Matejckava // Cas. Lek. Ces. – 1977. – T. 116, № 14. – P. 436–437.

Функциональные нарушения иммунного ответа в разные периоды развития експериментальной пневмониии

О. А. Чугай

Аннотация. Одним из основных механизмов защиты дыхательной системы от патогенов является функционирование различных звеньев иммунной системы слизистых респираторного канала. В работе выявлено, что в условиях экспериментальной пневмонии у морских свинок происходило подавление клеточного звена иммунитета, которое выражалось снижением уровня Т-лимфоцитов в крови. В то же время выявлена активация гуморального звена иммунитета, что проявлялось повышением уровня В-лимфоцитов и циркулирующих иммунных комплексов в крови как в ранний, так и поздний период экспериментальной пневмонии.

Ключевые слова. *Экспериментальная пневмония, Т-лимфоциты, В-лимфоциты, циркулирующие иммунные комплексы.*