PINOCYTOSIS OF SERUM GLOBULINS BY MACROPHAGES

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Histiocytes (macrophages from the bone marrow of guinea pigs), cultivated in vitro, actively engage in pinocytosis of homologous and, in particular, heterologous immunoglobulins. Autologous immunoglobulins, capable of giving an intracellular specific reaction with phagocytosed typhoid bacteria, were found only in a few macrophages. Autologous immunoglobulins were detected in a large proportion of rabbit alveolar macrophages not cultivated in vitro.

The existence of tissue immunity without antibodies has been conclusively proved [1, 3, 6, 7, 12, 13]. However, it has been reported that antibodies must be present in the surrounding medium for intracellular destruction of bacteria in macrophages [14]. In cases when specific globulins circulate in an immune organism, it is difficult to make a differential study of the "humoral" and "cellular" factors of immunity. In such cases a simplified microorganism–phagocyte system is widely employed in vitro, in which the cells of an immunized donor are first washed in order to prevent any opsonizing action of the serum. However, after the discovery of the phenomenon of pinocytosis, it appears that this method is not effective, because the globulin undergoing pinocytosis is located intracellularly.

In an earlier paper the writers described the ability of macrophages to carry out pinocytosis of serum globulins [5]. In the investigation described below, pinocytosis of immunoglobulins by macrophages in vitro and in vivo was studied.

EXPERIMENTAL METHOD

Macrophages (histiocytes from the bone marrow of guinea pigs) were cultivated in vitro on cover slips in medium No. 199 with 20% inactivated bovine serum [4]. During cultivation, the precursor cells underwent transformation into macrophage-histiocytes [2]. Alveolar macrophages were washed out of the lungs taken from the thorax of an exsanguinated rabbit. The suspension of cells was centrifuged 3 or 4 times in medium No. 199 with 2% inactivated bovine serum, after which the supernatant did not give a precipitation reaction with antiserum against rabbit globulins. In comparative experiments, a culture of transplantable fibroblasts (L strain) also was used. Films of alveolar macrophages and monolayer cultures of histiocytes and fibroblast, fixed in ethanol, were stained by direct and indirect luminescence-serologic methods for detecting intracellular (pinocytosed) globulins. The specimens were then mounted in a mixture of glycerol with physiological saline. The ML-2 microscope, with FS-1-2 energizing filter and No. 1 (ZhS-18-2 and ZhZS-19-1) shut-off filters, were used for luminescence-microscopic examination of the specimens. For photomicrography, 90×1 and 25-FL (oil immersion) objectives, a Homal 5× ocular, and foto-250 or RF-3 film were used.

The luminescent sera labeled with fluorescein isothiocyanate and the dry globulins (predominantly γ globulins) of rabbit, horse, and guinea pig were kindly made available by K. L. Shakhanina.

EXPERIMENTAL RESULTS

Pinocytosis of heterologous globulin was studied in the experiments of series I. Three-day cultures of histiocytes (guinea pig bone marrow macrophages) were incubated for 60-90 min in medium No. 199 with

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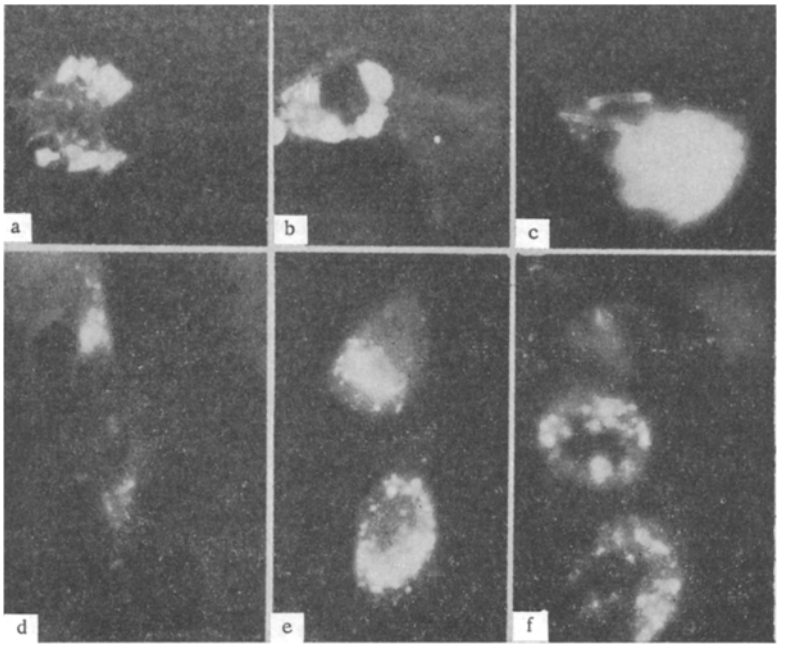

Fig. 1. Pinocytosis of globulins by macrophages. a, b) Bone marrow histiocytes, specific luminescence of heterologous (rabbit) globulin; c) specific luminescence of bacteria (0901) stained by labeled antiserum against guinea pig globulins; d) specific luminescence of homologous globulin in histiocyte-macrophages; e) specific luminescence of heterologous (rabbit) globulin in fibroblasts; f) specific luminescence of autologous globulin in 2 rabbit alveolar macrophages.

1-2 mg/ml rabbit globulin. Cultures of fibroblasts were treated in the same way. The cultures on cover slips were then carefully washed, fixed, and stained by the direct method with labeled ass antiserum against rabbit globulins. This same serum was used to stain some specimens incubated with horse globulin.

Cultures of macrophages incubated with rabbit globulin were also stained with nonspecific labeled serum. In the specifically stained specimens more than 80% of the histiocyte-macrophages contained bright green luminescent inclusions, resembling droplets of different sizes, which joined together to form large conglomerations in the cell cytoplasm (Fig. 1a, b). By contrast to these cells, the fibroblasts contained much less pinocytosed globulin, which was detected by luminescence microscopy merely as punctate, specifically luminescent inclusions (Fig. 1e).

In a similar experiment, pinocytosis of homologous globulin by histiocyte-macrophages was observed (Fig. 1d).

The object of the experiments of series II was to detect autologous globulins in rabbit alveolar macrophages and in guinea pig histiocyte-macrophages. Many of the cells in films of alveolar macrophages, stained by the direct luminescence-serologic method, contained the required globulins (Fig. 1f). Recently immunoglobulins have been demonstrated in human macrophages by testing with erythrocytes sensitized with antibodies against IgG [9]. Antibodies also were found even earlier in extracts of alveolar macrophages [10], where they had evidently undergone pinocytosis because these cells did not produce antibodies in the recipient's body.

Attempts to detect autologous globulins in guinea pig histiocyte-macrophages cultivated in vitro did not yield convincing results when either the direct or the indirect staining method was used.

It is possible that histiocyte-macrophages obtained by transformation in vitro from bone marrow mononuclear cells no longer contained globulins on the 3rd-4th day of cultivation. The possibility likewise is not ruled out that traces of uniformly distributed globulin, when stained by the luminescence-serologic method, may give weak, diffuse luminescence which it would be difficult to accept as specific in the presence of even a very slight nonspecific background. To detect intracellular antibodies in such situations it was

decided to adsorb them specifically on the surface of a bacterial cell, and then to stain them with a specific antiprotein labeled serum.

Cultures of histiocyte-macrophages were obtained from the bone marrow of immunized guinea pigs in whose sera the titer of specific agglutinins against Salmonella typhi strain 0-901 was 1:320-1:640. On the 3rd-4th day of cultivation, washed macrophage-histiocytes were incubated with killed bacteria (0-901; $50-100\times10^6$ ml) in medium No. 199 without serum. After 1.5 h, when nearly all macrophages contained phagocytosed bacteria, the cultures were fixed and stained with labeled antiserum against guinea pig globulins. Control films of bacteria treated with this serum did not give specific luminescence in activating blue light, but phagocytosed bacteria gave a bright luminescence after staining with this same serum, indicating that specifically attached guinea pig globulins were present on the surface of the bacteria (Fig. 1c).

Hence, the use of a combination of phagocytosis and luminescence-serologic methods showed that globulins (antibodies), after undergoing pinocytosis, can react specifically with intracellular antigen, in this case with phagocytosed bacteria. However, unlike alveolar macrophages, the histiocyte-macrophages obtained in vitro contained globulins very rarely (0.5-2% of cells), although the source of nearly all macrophages in the body is bone-marrow cells [8, 11, 15]. This difference in the globulin content is evidently due to the fact that in the first case the macrophages were formed in vitro [2] in the absence of autologous globulins, while in the second case they were formed in vivo.

This investigation thus evidently demonstrates the need for testing macrophages for their content of globulins when cellular immunity is studied, especially when the macrophages used were not obtained from bone marrow and were not cultivated in vitro. The possible role of pinocytosis of immunoglobulins in the mechanism of passive immunity cannot be ruled out.

LITERATURE CITED

1. A. T. Kravchenko and N. V. Galanova, The Third Factor of Acquired Immunity [in Russian], Moscow (1948).
2. E. A. Luriya, O. V. Chakhava, and A. Ya. Fridenshtein, Tsitologiya, No. 1, 115 (1966).
3. V. D. Solov'ev, Vopr. Virusol., No. 5, 580 (1964).
4. O. V. Chakhava and A. G. Goryunova, Antibiotiki, No. 6, 507 (1965).
5. O. V. Chakhava, J. Reticuloendothel. Soc., 3, 355 (1966).
6. S. S. Elberg, P. Mascarenhas, and J. Fong, Proc. Soc. Exp. Biol. (New York), 115, 879 (1964).
7. R. Esterly and A. M. Dannenberg, J. Reticuloendothel. Soc., 4, 428 (1967).
8. R. van Furth and Z. A. Cohn, J. Exp. Med., 128, 415 (1968).
9. H. Huber, S. D. Douglas, and H. H. Fudenberg, Immunology, 17, 7 (1969).
10. W. B. Hunt and Q. N. Myrvik, J. Immunol., 93, 677 (1964).
11. K. Lennert and L.-D. Leder, in: The Reticuloendothelial System, Otsu (1964), p. 25.
12. G. B. Mackaness, J. Exp. Med., 120, 105 (1964).
13. J. W. Osebold and R. di Capua, J. Reticuloendothel. Soc., 4, 428 (1967).
14. D. Rowley, Nature, 181, 1738 (1958).
15. M. Virolainen, Exp. Hematol., 15, 116 (1968).