INTERACTION BETWEEN INFLUENZA VIRUS AND MACROPHAGES DURING REALIZATION OF THE IMMUNE RESPONSE

T. Ya. Dubrovina, G. F. Leont'eva, I. E. Meshcheryakova, R. Ya. Polyak, and A. A. Smorodintsev*

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Interaction of noncellular and bacterial antigens with macrophages is generally accepted as an obligatory trigger stage of the immune process [12]. The functional role of macrophages in the development of antiviral immunity in connection with the great diversity of possible variants of response of these cells with different viruses has received much less study [6]. Macrophages sensitive to viruses promote their active reproduction [10]; in other cases viruses penetrate passively into the cytoplasm of macrophages during pinocytosis of virus particles or phagocytosis of virus-infected cells [3, 5]. Viruses can be selectively adsorbed on the outer membrane of macrophages [15]. In all these cases a different effect is produced on the functional state of the macrophages and on their role in the permissible preparation of virus supernatants or in induction of specific information received on an antigen-reactive clone of T-lymphocytes.

The object of this investigation was to study the role of macrophages in the stimulation of anti-influenzal humoral immunity, taking into account the functional state of the cells and the ability of the virus to realize its own genetic information in them.

EXPERIMENTAL METHOD

Allantois influenza A/Leningrad/538/74 (H3 N2) virus was used. Aggregated forms of the virus were obtained by adsorption of 10^5 EID_{50} of virus on hen's erythrocytes ($3.5 \cdot 10^7$ cells) and by adsorption of virus in a homogenate of chorioallantoic membranes of 12-day chick embryos, removed 16 h after infection with 10^3 EID_{50} of virus, on subcellular structures.

The infectious activity of the virus was determined by titration on developing chick embryos.

Pinocytes and monolayer cultures of peritoneal macrophages of Wistar rats and CBA mice were grown in an atmosphere with 5% CO_2 in medium No. 199 with 5% neonatal calf serum or 1% bovine albumin and 5 units/ml of heparin [4].

The reproductive activity of the virus in macrophages was studied in high and low multiplicity of infection (1-10 and 0.01-0.005 EID_{50} per cell, respectively). After contact for 1 h the virus which had not been adsorbed and had not penetrated into the cytoplasm was removed with type-specific serum and washed repeatedly with maintenance medium No. 199. The cells were disintegrated at various times after infection and infectious virus determined in the homogenate.

Detection of synthesis of virus-specific RNP-structures in the macrophages was studied at a high multiplicity of infection. After removal of nonpenetrating virus the cells were incubated at 37°C for 1 h and transferred to Eagle's medium with ^3H-uridine (10 μCi/ml) as radioactive RNA precursor. After incubation for 5 h at 37°C the unincorporated label was carefully washed out with cold buffered physiological saline. The RNP-structures were isolated by a sedimentation method [7], using the SW27 rotor of a Beckman L-75 centrifuge.

*Academician of the Academy of Medical Sciences of the USSR.

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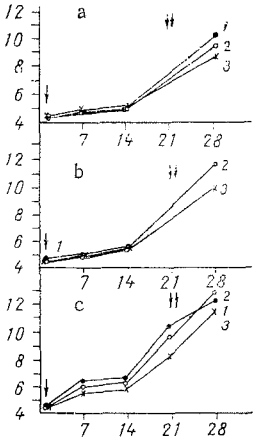

Fig. 1. Dependence of antibody formation on degree of dispersion of native influenza A virus preparation used as antigen. Virus in doses of 3 log EID_{50} (a), 5 log EID_{50} (b), and 7 log EID_{50} (c) was injected intraperitoneally into rats (arrow). All animals received a second (two arrows) intraperitoneal injection of 7 log EID_{50} of virus. Abscissa, days; ordinate, titer of serum (in $\log_2$); values given are reciprocals. 1) Allantoic virus; 2) virus adsorbed on erythrocytes; 3) virus adsorbed on structures of sensitive cells of chorioallantoic membranes.

Rats were immunized with intact or aggregated virus by a single intraperitoneal injection in a volume of 2 ml. Serum antibodies were determined in the neutralization of infectious activity of the virus for chick embryos test, using one dose of virus and successive two-fold dilutions of serum.

EXPERIMENTAL RESULTS

In previous investigations in the writers' laboratory [3, 5] activation of phagocytosis of macrophages was found on contact with aggregated forms of influenza virus, with the detection of considerably higher concentration of infectious activity in the cells. To study the relationships between the activity of phagocytosis and intensity of the immune response the animals were immunized with different variants of native virus; relatively monodispersed allantoic virus or virus aggregated on cells. Comparison of the dynamics of synthesis of serum antibodies 7 and 14 days after single immunization showed that the intensity of immunity depended on the dose of virus injected (Fig. 1). The quantitative indices of the antibodies and the dynamics of their accumulation did not depend on the degree of dispersion of the preparation. The formation of immunologic memory, studied relative to the intensity of antibody synthesis after repeated intraperitoneal injection of allantoic influenza virus 3 weeks after primary immunization also was comparable when different forms of virus antigen were administered. These results revealed differences in the character of the antigenic stimulus in native or inactivated virus; according to available evidence [1], the latter in the aggregated state possessed stronger immunogenic properties. One possible cause of the absence of correlation between the intensity of the immunologic reaction and the physical form of the native virus used could be functional heterogeneity of the macrophages [14], in the presence of which different clones of macrophagal cells respond-during the process of induction of antibody formation and during phagocytosis of cells infected with virus. Meanwhile, the possibility must not be overlooked of reproduction of native virus in the macrophages, which would lead to synthesis of virus polypeptides possessing antigenic specificity and would increase the antigenic stimulus. This hypothesis ought to be tested, because there is evidence of virus-specific synthesis in the macrophages of mice infected with influenza virus [2, 9, 13].

On the basis of these observations a role of macrophages in the induction of antibody synthesis in response to injection of native influenza virus can be postulated (Table 1). In experiments with adoptive trans-

TABLE 1. Role of Macrophages of CBA Mice in Formation of Humoral Immunity against Influenza

Cells injected into irradiated mice		Number of positively reacting sera	Total number of sera	Mean geometric antibody titers	Positive seroconversion, %
B-lymphocytes + T-lymphocytes	macrophages				
—	—	2	15	<2	0
+	+	12	15	11,3	80
+	—	0	11	0	0

Legend. In experiments, native influenza A virus was injected intraperitoneally into sublethally irradiated mice in a dose of $10^{8.5}$ EID_{50} and T-lymphocytes + B-lymphocytes or the total pool of spleen cells were injected in a dose of 35×10^6 cells taken from intact syngeneic mice.

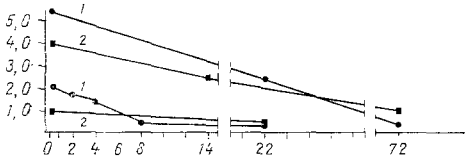

Fig. 2

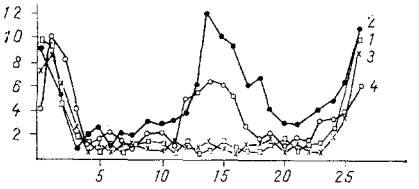

Fig. 3

Fig. 2. Influenza A virus elution curves in nutrient medium and rat macrophages. Abscissa, hours after infection; ordinate, infectious activity (in log EID_{50}). 1) Extracellular virus; 2) virus in macrophages.

Fig. 3. Detection of RNP-structures of influenza virus in sensitive MDSK cells and rat macrophages. Cells infected with 1-5 EID_{50} per cell. RNP-structures detected 5 h later by sedimentation in sucrose density gradient (15-30%) with 1 M urea, SW27 rotor of Beckman L-75 centrifuge (23,000 rpm, 17 h). Abscissa, Nos. of fraction; ordinate, radioactivity of ^{3}H-uridine (in CPM $+10^3$); 1) intact MDSK cells; 2) infected MDSK cells; 3) intact macrophages; 4) infected macrophages.

fer of a pool of splenocytes from intact syngeneic animals into sublethally irradiated (500 R ones), synthesis of antibodies was detected only in the case of simultaneous injection of virus and spleen cells. If the macrophages were removed from the pool, no immunologic response developed whatsoever.

Reproduction of influenza virus was analyzed on a monolayer culture of rat macrophages. The ability of the native virus to penetrate into unstimulated peritoneal macrophages was judged from the results of titration of intracellular virus at different times after infection. At different distances after infection with a massive dose of virus or a low dose of virus, 3.5 and 1 log EID_{50} of virus respectively was found in the homogenate (Fig. 2). After culture for 72 h not only had no accumulation of virus taken place, but a decrease in its titer was observed, corresponding to the thermal inactivation curve of extracellular virus. This is in agreement with results obtained by other workers who have studied interaction between influenza viruses and mouse macrophages [3, 15]. In connection with a report of a significant increase in the accumulation of parainfluenza virus with a fall in the temperature of culture of macrophages [8], it was decided to study the possibility of more active reproduction of influenza virus in rat macrophages between temperatures of 28 and 40°C. The results showed that under these conditions also no reproduction of the virus took place, and intracellular death took place in full agreement with the rate of this process outside the cells, at each of the chosen temperatures.

The results of the virologic investigation thus showed that macrophages of Wistar rats do not support reproduction of influenza A virus and that they are nonpermissible for synthesis of the virus by the cell system.

To clarify the events taking place in macrophages infected with influenza virus, a series of experiments was carried out using molecular-biological methods of detection of virus-specific syntheses of RNP-structures. It was found that 5 h after infection of macrophages with influenza virus, structures analogous in characteristics of RNP detectable in a highly sensitive culture of transplantable MDSK cells (Fig. 3), appeared in them. Consequently, partial realization of the genome, leading to RNP synthesis, took place in the macrophages in the absence of synthesis of infectious virus. Further analysis of ^{14}C-labeled amino acids of virus-specific polypeptides, undertaken in parallel experiments in infected sensitive cells and macrophages by electrophoresis in polyacrylamide gel [11], revealed a disturbance of translation in the synthesis of virus polypeptides.

An analysis of the characteristics of interaction of native influenza A virus with rat and mouse macrophages thus showed that macrophages take part in the induction of synthesis of anti-influenzal antibodies. The magnitude of the antigenic stimulus correlated with the concentration of virus injected, but was independent of the degree of dispersion of the antigen from native virus. This indicated that the inductive phase of the immune response was independent of the phagocytic activity of the macrophages. The results of the virologic and molecular-biological analysis showed resistance of rat macrophages to virus reproduction, although synthesis of virus-specific RNP-structures with disturbance of the process of translation of virus-specific polypeptides was discovered in them. Consequently, the absence of any difference in the intensity of the immunologic reaction when aggregated or monodispersed forms of virus were used was not due to additional accumulation of virus antigen. The results indirectly support the view [14] that the process of active phagocytosis and function of specific processing of the antigen for stimulation of specific lymphocyte proliferation belong to different classes of macrophages or they are spatially separate from each other in the same cell.

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