PROTECTIVE ACTION OF CYTOTOXIC LYMPHOCYTES DEPENDING ON ANTIGENIC DETERMINANT COMPOSITION OF INFLUENZA VIRUS HEMAGGLUTININ

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There is no denying the importance of cell-mediated reactions in antiviral immunity at the present time. We know that cytotoxic T lymphocytes (CTL) and also normal killer cells [2, 9] can recognize and lyse virus-infected cells and thus limit the spread of infection. In many virus diseases the action of CTL is regarded as one of the leading mechanisms in the elimination of infected cells from the body [1, 10].

The infectivity of the influenza virus is connected with its surface component (hemagglutinin), antibodies to which are also responsible for the protective effect. As regards the key factors of immunity, recent data are evidence that in relation to influenza viruses the CTL population, like antibodies, is polyclonal and that there exist both cross-reactive CTL, found mainly in man, and strain-specific CTL [1, 13]. The target for the first type consists of nonstructural proteins synthesized by the virus of the cell (NS1, NS2), core proteins of the virion (nucleotide, various polymerases), and certain proteins of hemagglutinin [13]. The specificity of these CTL is thus manifested within the same serologic type. On the other hand, it has been shown by genetic engineering methods that different CTL can distinguish between hemagglutinins of subtypes H1, H2, and H3, i.e., their specificity is narrower. In the opinion of these authors [12, 14, 15], such CTL are characterized by strain specificity.

However, investigations [4-7, 11] have shown that the hemagglutinin of the influenza virus, of both type A and type B, contains in its composition at least three epitopes, namely type-, group-, and strain-specific antigenic determinants. Specific antibodies against hemagglutinin epitopes possess a marked protective action.

The aim of this investigation was to study the protective action of CTL of varied specificity in influenzal infection, depending on the similarity or difference between hemagglutinin determinants of viruses used to immunize and infect animals. The experiments made use of the adoptive transfer phenomenon. Altogether 1500 animals were used in the experiments.

EXPERIMENTAL METHOD

Male CBA mice (kkkkkk haplotype) were used as the biological model. Mice weighing 16-18 g served as donors and mice weighing 12-14 g as recipients.

The following type A influenza viruses with the H3N2 antigenic formula were used for immunization: A/Aichi 2/68 [1.2.3]; A/Tokyo 1/75 [1.5.7.]; A/England 42/72 [1.4.5]; A/Bangkok 1/79 [7.8.10]; A/Shanghai 31/80 [7.8.11]. All these viruses were obtained from the Museum of Virus Cultures, D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR. The viruses were cloned by the limiting dilutions method and subjected to repeated passage in the allantoic sac of 9-day chick embryos. For immunization, the cloned virus was injected intraperitoneally in a volume of 0.5 ml.

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Adoptive Transfer. Mouse spleens obtained from animals immune to influenza viruses on the 6th day after immunization were homogenized in a glass homogenizer and filtered through four layers of Kapron gauze; after hemolysis of the erythrocytes in a 0.78% NH_4Cl solution the cells were washed in medium 199, centrifuged at 800-1000 rpm for 8-10 min, and then counted in a Goryaev chamber. Cells were injected in a dose of 10^8 into the caudal vein of recipient mice in a volume of 0.5 ml, after which the animals were infected on the same day with A/Aichi virus. Infected mice receiving splenocytes from nonimmune animals and from animals immunized with normal chick embryonic chlorioallantoic membrane (NCAM), and also infected mice not receiving splenocytes served as the control groups of animals.

To reproduce experimental influenza, we used A/Aichi 2/68 influenza virus, adapted to mice, and with an infectious titer by intranasal injection of 4.5 log LD_{50}/0.05 ml. Virus-containing allantoic fluid was injected in a dose of 5-10 LD_{50}/0.05 ml. The adoptive immunization effect was evaluated as the percentage of protection of the animals, determined by the difference between mortality in the experimental and control groups of animals. The results were subjected to statistical analysis by the Student–Fisher method.

Protection of the animals in the group receiving splenocytes from mice immune to Aichi virus and subsequently infected with the same virus, was 42%. Incidentally, in different experiments this figure varied between 30 and 42%. Thus with complete identity of the three antigenic determinants of hemagglutinin of the virus used for immunization and for realization of influenzal infection (determinants 1.2.3), the adoptive transfer effect was considerable. In the group of animals receiving splenocytes from donors immunized with England [1.4.5] or Tokyo [1.5.7] viruses and subsequently infected with Aichi virus [1.2.3], as Table 1* shows, the mortality of the mice was 100%. Thus the presence of only one common antigenic determinant in the composition of the hemagglutinins of viruses used to immunize and infect the animals gave no protection to the animals whatsoever. Of course no protective action of CTL likewise was observed in the group of mice receiving splenocytes from animals immunized with influenza viruses Bangkok [7.8.10] or Shanghai [7.8.11], i.e., in the case when none of the three antigenic determinants was also common to the reacting virus [1.2.3].

In the control group of animals receiving splenocytes immune to NCAM, as Table 1* shows, no protective action likewise was observed and mortality in this group was 100%. In the other control group of mice, into which intact splenocytes were injected before infection, mortality of the animals was 96%. Finally, in the 3rd (control group of mice, which received no splenocytes at all and were simply infected), protection amounted to 8%. Our experiments showed that the titer of antibodies to Aichi virus in the sera of the recipient mice in the experimental and control groups was the same (in the CFT 1:20-1:80) on the 9th-21st day of infection. This is in agreement with the results obtained by other investigators and is evidence that under adoptive transfer conditions the protective action is due actually to the CTL and not to antibodies or helper T cells.

The results of these experiments thus show that CTL have no protective action under adoptive transfer conditions if one common antigenic determinant of hemagglutinin is present in the composition of the virus forming immunity and of the infecting virus. This distinguishes CTL from antibodies capable of protecting animals under similar conditions and at the same time enables individual hemagglutinin epitopes to be more finely differentiated [3]. Meanwhile, with complete identity of the antigenic determinants of hemagglutinins in the composition of influenza viruses used for immunization and subsequent infection of animals, besides antibodies, CTL are among the basic factors of antiviral immunity.

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