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SENSITIZATION TO INFLUENZA A_2 VIRUS IN COMBINED EXPERIMENTAL INFECTION

S. Gönczi

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Influenzal infection can significantly aggrevate bronchial asthma or provoke its onset. Vaccination against influenza can also exacerbate asthma. After viral infection, resistance to secondary Gram-negative infection is reduced. Experimental data on the mutual effect of these two infections are contradictory [1, 3, 5, 7, 9-12].

The aim of this investigation was to develop a model of combined immunization to influenza virus and *Haemophilus influenzae* (HI), for the latter is very often found in healthy people. The effect of infection due to HI on the degree of sensitization to influenza virus was studied.

EXPERIMENTAL METHOD

Noninbred guinea pigs weighing 250-300 g were used. In the experiments of series I a method of viral vaccination of the guinea pigs was used and the doses of viral antigens not inducing a reaction in intact animals (n = 38) were determined in various tests. Strain A_2 Victoria 35/72 H_3N_2 was used as the influenza vaccine. After preliminary propagation on chick embryos the strain of influenza virus was adapted (N. A. Andreeva) to guinea pigs by intranasal infection. The virus was reisolated from nasal washings of the animals on the 4th-5th day after infection. The animals were immunized with influenza vaccine once, intra-

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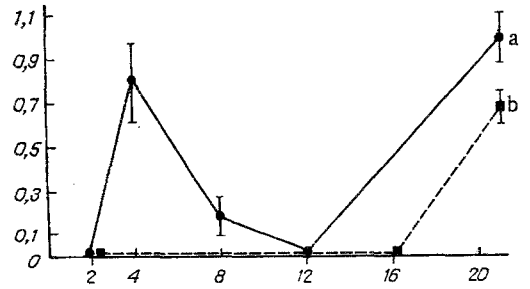

Fig. 1

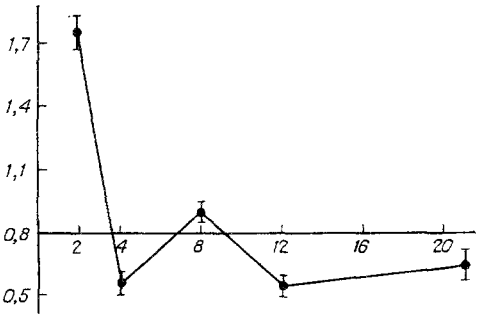

Fig. 2

Fig. 1. Time course of delayed skin tests after injection of viral antigen into animals immunized with influenza virus: a) influenza virus allergen in a dilution of 1:20: b) in a dilution of 1:40. Here and in Fig. 3: abscissa, time of investigation (in weeks).

Fig. 2. Time course of LMIT after injection of virus antigen into animals immunized with influenza virus. Abscissa, time of investigation (in weeks); ordinate, migration index.

nasally. The nasal passages were washed out beforehand with physiological saline, treated with 0.5% Procaine solution, after which the vaccine was instilled in a titer of 1:128 in the hemagglutination test, in a dose of 0.3 ml.

In accordance with a model of immunization developed previously, living HI cells (serovar B, biotype 1, No. 11, 1981) were injected into the footpads of the guinea pigs in doses of $4 \cdot 10^9$ and $4 \cdot 10^{10}$ bacterial cells (b.c.).

In series II combined sensitization to influenza virus and HI was carried out. For this purpose the guinea pigs were sensitized by four different schemes (10 guinea pigs by each scheme): animals of group 1 were first immunized with HI in a dose of $4 \cdot 10^{10}$ b.c. and 1 month later with influenza virus; animals of group 2 were immunized first with HI in a dose of $4 \cdot 10^9$ b.c., and with influenza virus 1 month later; animals of group 3 were immunized first with virus, and 1 month later with HI in a dose of $4 \cdot 10^{10}$ b.c.; animals of group 4 were immunized simultaneously with HI in a dose of $4 \cdot 10^9$ b.c. and with influenza virus. The following allergens were used in the various immunologic tests: vaccine strain A_2 Victoria and antigen S (obtained by N. L. Cheretenko from museum strain A_2 Port Chalmers and from strain A_2 42/75, isolated from patients in Alma-Ata). Antigen S is a soluble component of the virus obtained by ether extraction, and with a degree of purity with respect to protein 200 times or more greater than the original allantoic fluid. For the control, all the tests were carried out on guinea pigs with allantoic fluid with the same dilutions of virus antigens. After immunization for 20-21 days the animals' general condition was studied monthly and specific immunologic tests carried out. For the intradermal tests, 0.1 ml of living influenza vaccine with a titer of 1:128 in the hemagglutination test (HT), in dilutions of 1:20 and 1:40 was injected into each animal. The results of the tests were read after 3, 24, and 48 h. The leukocyte migration inhibition test (LMIT) was carried out by the method in [6] in capillary tubes placed in wells in standard sterile plastic plates. Cells emerging from the capillary tubes and settling on the floor were resuspended and the number of leukocytes in the medium determined after hemolysis of the erythrocytes and with 2% saponin solution on an automatic apparatus of Pikoskel (Hungary) type for counting blood cells. The LMIT was considered to be positive if the migration index (MI) was below 0.8. Influenza virus allergen was used for LMIT in solutions of 1:40 and 1:80. The passive cutaneous anaphylaxis test (PCAT) was performed by the method in [13]. Pale guinea pigs were given an intradermal injection of 0.1 ml of the sera of sensitized and intact animals 24-48 h before intravenous injection of the antigen in a volume of 0.5-1.0 ml, with titers of 1:640 and 1:1280 in the HT, mixed with an equal volume of 1% solution of Evans' blue. The results were read after 15-30 min. The class of IgE antibodies was confirmed in some experiments by heating the sera to 56°C for 2 h. The heated sera could no longer induce PCAT. For the cutaneous anaphylaxis test (ovary's active test) on immunized animals antigen S was used in a dose of 0.1 ml of the whole preparation. The animals were given an intravenous injection of 0.5 ml of 1% solution of Evans' blue 5 min later. The reaction was negative in intact animals.

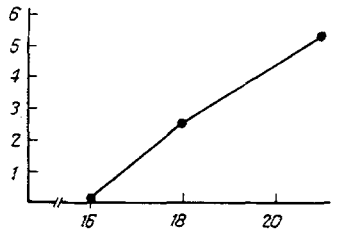

Fig. 3

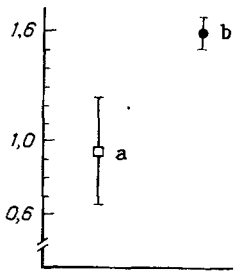

Fig. 4

Fig. 3. Time course of PCAT after injection of virus antigen into animals immunized with viral vaccine.

Fig. 4. Delayed skin tests after infection of animals with HI in a dose of $4 \cdot 10^9$ and with A_2 virus. a) HI; b) HI + A_2 virus.

EXPERIMENTAL RESULTS

The general conditions of the animals after vaccination with influenza virus was unchanged in the case of monosensitization. A small discharge from the nose of the guinea pigs was observed. Titers of the virus in the washings were between 1:2 and 1:64 in the HT.

It will be clear from Fig. 1 that delayed skin tests after injection of virus antigen became positive 4 weeks after vaccination. At the 2nd and 3rd times of investigation the tests were virtually negative. At the last time the skin tests became positive again. The results of LMIT confirmed the formation of cellular reactivity (Fig. 2). LMIT was positive 4 weeks after vaccination with influenza vaccine (MI = 0.64 ± 0.07). At the last time of the experiment the reaction became positive again, evidence that delayed-type hypersensitivity (DTH) to influenza virus persisted for a long time. Because of their intracellular localization, viruses activate the T-cell mechanism of immunity. This has been proved in many experimental models with respect to Coxsackie and herpes simplex viruses [14]. The state of local immunity and local sensitization is of definite importance for the virus. We know that intranasal immunization induces DTH more effectively than parenteral. Influenza virus antigens can induce an IgE response and the formation of antigen-specific IgE [15].

To study the formation of immediate-type hypersensitivity (ITH) the PCAT was performed on animals at different times of the experiment. The reaction was positive 18 weeks after immunization (Fig. 3). At the last time enhancement of PCAT was observed. After intravenous injection of the viral allergen, large spots, quickly turning blue, appeared. To test the specificity of PCAT to viral antigen, the active cutaneous anaphylaxis test (ACAT) was carried out. Antigen S was injected intradermally into the sensitized animals: the reaction was strongly positive. ACAT and PCAT with allergen (allantoic fluid in dilutions of 1:20 and 1:40) were negative.

A combination of two infections may have a considerable influence on the state of sensitization to one of them or to both. We studied their effect of sensitization to the virus. Simultaneous injection of the antigens caused significant enhancement of the delayed skin tests. The results of LMIT confirmed the formation of DTH, especially at the first time of observation (MI = 0.57 ± 0.02; Fig. 4).

Subsequent infection with influenza virus after infection with HI in a dose of $4 \cdot 10^{10}$ b.c. was accompanied by the development of sudden weakness and profuse discharges from the nose. Influenza virus was found in the nasal washings in a titer of 1:640 or 1:1280 in the HT. With this combination of infection, PCAT became positive 12 weeks after vaccination with influenza virus.

The general state of the animals infected first with HI in a smaller dose ($4 \cdot 10^9$ b.c.) was unchanged after viral vaccination. PCAT became positive in the guinea pigs of this group at the earliest time, only 4 weeks after viral infection.

Preliminary infection of the animals with influenza virus, followed by infection with HI in a dose of $4 \cdot 10^{10}$ b.c. did not potentiate the formation of DTH or ITH to viral allergens.

There is information in the literature on the potentiating action of viruses on allergic reactions. According to the observations of Alekseeva et al., influenza virus was a stronger

antigen than ragweed pollen. They showed, for instance, that simultaneous injection of antigens of influenza A_2 virus and staphylococci accelerates the appearance of DTH to the viral antigen [2, 4].

According to our own observations monovalent vaccination of guinea pigs with a living culture of influenza A virus caused the development of DTH toward the end of the first month. Animals of this group developed immediate cutaneous sensitivity in the late stages of the investigation.

After simultaneous infection with HI and influenza virus enhancement of the delayed skin tests was observed. After successive infection under certain conditions the development of cutaneous anaphylaxis was accelerated in the PCAT. In this case, several mechanisms may perhaps be involved. The effect of immunization with HI on vaccination with influenza virus depends on the order of infection and on the dose of the antigens. The capsular form may perhaps possess adjuvant properties and may stimulate the development of sensitization to influenza virus.

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