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PREPARATION OF MONOSPECIFIC IMMUNOGLOBULIN AGAINST HUMAN LEUKOCYTIC INTERFERON

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Donkeys were immunized regularly at 7-day intervals with human leukocytic interferon, injected subcutaneously in a dose of 1600 units in 10 ml. Interferon-neutralizing antibodies were found in a titer of 1:128-1:256 in the sera of the animals after 38-40 immunizations. As a result of continued immunizations the titer of these antibodies rose considerably. Parallel tests revealed antibodies against components of the system in which the interferon was obtained. Donkey interferon plasma was prepared by plasmaphaeresis and an anti-interferon immunoglobulin was isolated from it by precipitation with ammonium sulfate at 50% saturation. Anti-interferon immunoglobulin was freed from contaminating antibodies by affinity chromatography on a combined immunosorbent.
KEY WORDS: *interferon; anti-interferon serum; monospecific immunoglobulin.*

Several recent investigations have yielded evidence of the antigenic properties of human interferons [2, 9, 10]. Preparation of antibodies against interferon presents the investigator with wide opportunities. To begin with, antibodies against interferon are necessary in order to study its antigenic properties and serologic specificity [7, 9]. Second, chromatographic separation of interferon and impurities by means of highly specific antibodies leads to a considerable increase in specific activity. Some investigations have already been carried out in this direction, on mouse [11, 12] and human [5, 6] interferons. The possibility of using immunologic methods to determine and titrate interferons is of great interest [15].

It has recently been shown that interferon is an important mediator of immunity [3, 13] and that, evidently, antibodies against interferon can be used to study the mechanism of formation of immunologic reactivity. The view has been expressed that hyperproduction of interferon *in vivo* leads to the development of autoimmune and allergic diseases [15]. In this connection antibodies against interferon may perhaps be used in clinical practice in order to produce immunodepression, and also as a component of the system of treatment of allergic and auto-immune diseases [4, 14, 15].

The object of the present investigation was to obtain a highly specific anti-interferon preparation (anti-interferon immunoglobulin) for immunologic and immunoclinical investigations and, in addition, to accumulate a sufficient amount of this material for clinical trials.

EXPERIMENTAL METHOD

Animals. Three donkeys each weighing 250-300 kg were used for immunization.

Interferon. Human leukocytic interferon with an activity of about 32 units/ml was provided by the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR.

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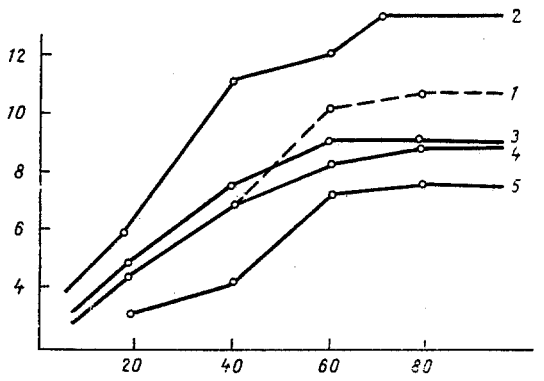

Fig. 1. Antibodies in serum of donkey immunized with human leukocytic interferon: 1) antibodies against human leukocytic interferon; 2) antibodies against human serum proteins; 3) antibodies against antigens of human leukocytes; 4) antibodies against virus; 5) antibodies against proteins of allantoic fluid. Abscissa, number of immunizations; ordinate, titer of antibodies ($-\log_2$).

Immunization. Each animal received regular subcutaneous injections at intervals of 7 days of 1600 units of human leukocytic interferon.

Determination of Interferon-Neutralizing Antibodies. The presumed immune serum was diluted successively from 1:2 to 1:4096 and an equal volume containing 10 units of human leukocytic interferon was added to each dilution; the mixture was incubated for 1 h at 37°C and then for 24 h at 4°C. The contents of each tube were transferred to a monolayer of a continuous culture of human embryonic cells and the cells were then incubated at 37°C for 24 h; next, after removal of the liquid from the tubes, the residual contents were covered with a layer of 100 CPD_{50} of the test virus (vesicular stomatitis virus). A similar parallel test with normal donkey serum was used as the control. The reaction was read after 24 h. The titer of donkey anti-interferon serum was taken to be the highest dilution which still neutralized the antiviral action of 10 units of human leukocytic interferon.

Determination of Contaminating Antibodies. Besides antibodies to interferon, the sera of the immunized animals were found to contain antibodies against components of the system in which the human leukocytic interferon was obtained, namely: against human serum proteins, soluble antigens of human leukocytes, Newcastle disease virus, the inducer of interferon, and proteins of the allantoic fluid of the chick embryos in which the virus was cultured. These antibodies (contaminating antibodies) were investigated by the passive hemagglutination test (PHT) with formalinized sheep's red blood cells sensitized with tannin. The PHT was performed in a Takachi microtitrator with 0.2% normal rabbit serum as the supporting medium.

Preparation of the Donkey Anti-interferon Plasma. After detection of antibodies against human leukocytic interferon in the blood of the immunized animals the immune donkey plasma was prepared by the method of plasmaphaeresis [1] and stored in plastic bags at -20°C.

Isolation of Anti-interferon Immunoglobulin and Its Purification from Contaminating Antibodies. The γ-globulin fraction was separated from the donkey anti-interferon plasma by precipitation with ammonium sulfate at 50% saturation [8] and the anti-interferon immunoglobulin was purified from contaminated antibodies by the method of affinity chromatography on a combined immunosorbent, which included in its composition antigens, antibodies against which have been found in donkey anti-interferon sera [5]. A commercial preparation of CNBr-activated Sepharose 4B, from Pharmacia, Sweden, was used as the insoluble basis for the immunosorbent. The dry preparation of activated Sepharose was wetted in 1×10^{-3} M HCl and washed with this solution to remove the preservatives, after which it was transferred to a glass chromatographic column. The following antigens were added: whole human serum, homogenate of human leukocytes obtained by ultrasonic treatment of a suspension of 200 million

TABLE 1. Titer of Antibodies in Donkey Anti-interferon Immunoglobulin before and after Absorption

Antibodies	Normal donkey globulin	Anti-interferon immunoglobulin	
		before absorption	after absorption
Against human serum proteins	1:4	1:16384	1:8
Against antigens of human leukocytes	1:2	1:1024	1:4
Against Newcastle disease virus	1:2	1:1024	1:4
Against proteins of allantoic interferon	1:2	1:512	1:4
Against human leukocytic interferon	1:2	1:2048	1:2048

leukocytes in 5 ml physiological saline (the 150W ultrasonic disintegrator from MSE, England) for 10 min with the instrument working at maximal output, Newcastle disease virus (10^9 PFU/ml), and allantoic fluid from chick embryos. All the above components were first dialyzed against buffered physiological saline, pH 7.2.

The material in the column was mixed by slow rotation for 18 h at room temperature, and to block the active groups of Sepharose not bound with proteins it was treated with 1 M monoethanolamine, pH 8.0 (BDH, England). The immunosorbent was washed successively with 0.1 M acetate buffer, pH 4.0, and 0.1 M borate buffer, pH 8.4, and then transferred to buffered physiological saline, pH 7.2. The rate of flow of the buffer solutions through the column with the immunosorbent did not exceed 40 ml/h.

The donkey anti-interferon immunoglobulin was passed through the column containing the immunosorbent at a speed of 10 ml/h at room temperature, and then concentrated to its original volume by ultrafiltration under pressure on PSED filters (Millipore, USA). The immunosorbent was regenerated with 0.1 M acetic acid containing 1 M NaCl, transferred to buffered physiological saline with 0.1% sodium azide, and kept at 4°C. Under these conditions the combined immunosorbent could be used to purify donkey anti-interferon immunoglobulin in 6 to 8 experiments.

EXPERIMENTAL RESULTS

Data reflecting the dynamics of antibody formation in donkey serum No. 1 in the course of immunization with human leukocytic interferon are shown in Fig. 1. The corresponding parameters for the other two animals did not differ in principle from those given in Fig. 1.

Interferon-neutralizing antibodies were found after 38-40 injections of the preparation in a titer of 1:128-1:256. As a result of continued immunization the titer of these antibodies increased to 1:2048-1:4096 and was maintained at this level by periodic reimmunization (Fig. 1).

Contaminating antibodies were discovered in the donkey sera after 7-8 immunizations in various titers, and their activity rose considerably during continued immunization.

By means of the plasmaphaeresis method 1 liter of immune plasma could be obtained every week from each donkey. Altogether more than 50 liters of donkey anti-interferon plasma were prepared.

Absorption of donkey anti-interferon immunoglobulin on the combined immunosorbent led to removal of its contaminating antibodies. The titer of these antibodies after passage of the preparation through the column with the immunosorbent was virtually indistinguishable from the titer of normal donkey globulin. It is interesting to note that the specific activity of the preparation was unchanged after absorption (Table 1).

As a result of immunization of donkeys with human leukocytic interferon an anti-interferon plasma with high specific activity (1:2048-1:4096) was thus obtained. Besides interferon-neutralizing antibodies, the serum of the immunized animals was also found to contain

contaminating antibodies, which had to be removed in order to increase the specificity of the anti-interferon immunoglobulin. The method of affinity chromatography on combined immunosorbent used for this purpose enabled all the contaminating antibodies discovered to be removed simultaneously without any decrease in the specific activity of the preparation.

Considering the results of previous investigations into the effect of anti-interferon serum on some aspects of the immune response [13, 14] there is reason to suppose that, with the availability of this highly specific anti-interferon immunoglobulin the various biological properties of interferon can be studied more fully.

Anti-interferon immunoglobulin is being used by the writers at the present time to study the role of interferon in immunity [14]. Preliminary data showing that the anti-interferon immunoglobulin obtained as described above is an active substance for the treatment of various allergic and autoimmune diseases have already been obtained [15].

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