DETERMINATION OF HUMAN LEUKOCYTIC INTERFERON BY A MICROFLUOROMETRIC IMMUNOLOGICAL METHOD

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The possibility of quantitative determination of human leukocytic interferon by means of anti-interferon antibodies labeled with fluorescein isothiocyanate (FITC) was studied. As a result of prolonged immunization of a donkey with a preparation of human leukocytic interferon and subsequent fractionation and immunosorption, a donkey antiinterferon immunoglobulin with high specific activity was obtained. This immunoglobulin was labeled with FITC and used for quantitative determination of human leukocytic interferon in direct and indirect immunologic inhibition of fluorescence tests. The titers of the various preparations of human leukocytic interferon in the inhibition of fluorescence test were comparable with the titers of these same preparations in the test of inhibition of virus cytopathic action by interferon.

KEY WORDS: interferon; anti-interferon immunoglobulin; quantitative immunofluorescence.

Methods currently used to determine interferon are somehow or other connected with the assessment of its antiviral action. All these methods are based on comparison of the intensity of replication of a virus or its components in cells treated with interferon and in analogous control cells [7].

The authors have attempted to determine interferon quantitatively on the basis of its ability to interact with specific antibodies, which many workers have demonstrated [3, 4, 9, 10]. The method of quantitative immunofluorescence in two modifications — the direct and indirect fluorescence inhibition tests [8] — was used for this purpose.

The hypothetical mechanism of this test as used in the titration of human leukocytic interferon is that different quantities of test interferon interact with a standard dose of fluorochrome-labeled antibodies (anti-interferon immunoglobulin). This mixture is then

TABLE 1. Titers of Interferon and Control Preparations in Direct and Indirect Fluorescence Inhibition Tests and Virus Cytopathic Action Inhibition Test

Interferon preparation	Titer in tests ($-\log_2$ of dilution)		
	fluorescence inhibition		virus cytopathic action inhibition
	direct	indirect	
Concentrated interferon	8,33 (7—9) $n=6$	9,75 (9—10) $n=4$	9,8 (9—10) $n=5$
	$P<0,05$		
P-IF 98102	15,75 (15—16) $n=3$	17,75 (17—18) $n=4$	18 ($n=1$)
	$P<0,05$		
"Pseudo" interferon	2,7 (2—4) $n=7$	3,5 (3—4) $n=4$	3,0 (1—3) $n=4$
Mouse interferon	<1 $n=4$	<1 $n=4$	<1 $n=4$

Legend: 1) Mean values given, with limits of variation in parentheses; 2) n denotes number of observations.

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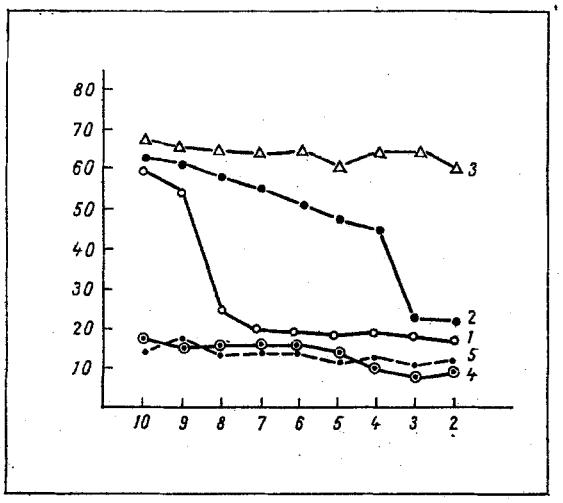

Fig. 1. Titration of human leukocytic interferon in the direct fluorescence inhibition test. 50 µl of 20% suspension of interferon-Con A-Sepharose was incubated with 25 µl FITC-labeled donkey anti-interferon immunoglobulin to which 25 µl human leukocytic interferon had been added (1). 25 µl of "pseudo" interferon (2), 25 µl mouse interferon (3); mixture of human leukocytic interferon (4) and "pseudo" interferon (5) with labeled anti-interferon immunoglobulin was incubated with Con A-Sepharose treated with normal rabbit serum. Abscissa, dilutions of interferon preparations ($-\log_2$); ordinate, intensity of fluorescence (in relative units).

incubated with a soluble polymer (Sepharose) on which the same interferon was adsorbed. The quantity of labeled antibodies capable of reacting with fixed interferon is inversely proportional to the quantity of test interferon added to it. By measuring the intensity of fluorescence of the Sepharose granules, the relative quantity of test interferon can be calculated.

1. Immunoglobulin against Human Leukocytic Interferon. To obtain antibodies against human leukocytic interferon, a donkey was immunized with a commercial preparation of this interferon produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology [1]. Interferon-neutralizing antibodies in the animal's serum were found 10 months after the beginning of immunization in a titer of 1:256 in the neutralization of the antiviral action of interferon test. As a result of continued weekly injections of the preparation, the antibody titer rose to 1:4096. A donkey anti-interferon plasma was prepared by plasmaphaeresis and kept at −20°C. Besides interferon-neutralizing antibodies, antibodies against the various components of the system in which the interferon was obtained (contaminating antibodies) also were found in the donkey's serum by the passive hemagglutination test. These were antibodies against human serum proteins, against soluble antigens of human leukocytes, against Newcastle disease virus — used to induce interferon — and against proteins of the allantoic fluid of the chick embryos. The globulin fraction was isolated from the donkey anti-interferon plasma by precipitation with ammonium sulfate at 50% saturation, and the anti-interferon immunoglobulin thus obtained was purified from the contaminating antibodies discovered by affinity chromatography on a combined solid-phase immunosorbent [3], and then with fluorescein methylisothiocyanate (FITC) [11]. As a result, labeled monospecific immunoglobulin against human leukocytic interferon with a titer of 1:2048 was obtained.

2. Immunologic Fluorescein Inhibition Test. Human leukocytic interferon adsorbed on Sepharose 4B, activated with concanavalin A (Con A-Sepharose 4B; from Pharmacia, Sweden) [6]

was used in this test. The interferon for addition to this carrier was dialyzed against 0.1 M accetate buffer, pH 6.0, containing 1 M NaCl, 10^{-3} M $MnCl_2$, 10^{-3} M $MgCl_2$, and 10^{-3} M $CaCl_2$, and added to the Sepharose in the ratio of approximately 5000 units to 10^6 granules. The mixture was incubated for 3 h at room temperature with constant mixing and then washed three times with the original acetate buffer. A 20% suspension of interferon-Con A-Sepharose in 0.1 M acetate buffer was used for the tests.

Direct Fluorescein Inhibition Test. FITC-labeled anti-interferon immunoglobulin was introduced in a dose of 25 µl into the wells of a Takachi microtitrator plate (Hungary) and serial double dilutions of the test interferon were added in a dose of 25 µl to each well. After incubation for 30 min at room temperature the mixture was transferred to plastic test tubes, to each of which 50 µl of the 20% suspension of interferon-Con A-Sepharose had previously been added. The material was incubated for 3 h at room temperature and washed three times with the original acetate buffer, after which samples were prepared for measurement of the intensity of fluorescence.

Indirect Fluorescence Inhibition Test. To 25 µl of unlabeled donkey anti-interferon immunoglobulin 25 µl of serial double dilutions of interferon was added, with mixing, just as in the direct test. After incubation for 30 min at room temperature the mixture was transferred to plastic test tubes, each containing 50 µl of a 20% suspension of interferon-Con A-Sepharose (1st layer). After incubation for 3 h at room temperature the material was washed with acetate buffer and 50 µl FITC-labeled rabbit serum against donkey globulin was added to each tube (2nd layer). After incubation for 3 h the material was washed three times with acetate buffer and samples were prepared for measurement of the intensity of fluorescence.

3. Preparation of samples and measurement of the intensity of fluorescence were carried out in the same way in the direct and indirect tests. To the residue of Sepharose granules in each test tube 50 µl of buffered glycerol was added (1 part glycerol to 1 part acetate buffer, pH 6.0), the suspension was transferred to a slide for the light microscope, a coverslip was applied, and the preparation was surrounded by an airtight border of paraffin to prevent drying. The intensity of fluorescence was measured on the ML-4 microscope with the FÉML-1 photometric attachment. Readings were made in arbitrary scale division units. The intensity of fluorescence of 10 granules was measured in each preparation.

4. Interferon and Control Preparations. The following were tested in the direct and indirect fluorescence inhibition tests: 1) a commercial concentrated preparation of human leukocytic interferon, produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology; 2) purified human leukocytic interferon, batch P-1F 98102, obtained from Dr. Cantell in Finland; 3) as a control of the specificity of the anti-interferon immunoglobulin, human leukocytic "pseudo" interferon, obtained in the same system as true interferon but excluding virus induction, and interferon induced in mouse L cells by Newcastle disease virus, were used; 4) as a control of the specificity of fluorescence, Con A-Sepharose treated with normal rabbit serum was used.

As Fig. 1 shows, addition of increasing quantities of human leukocytic interferon to FITC-labeled anti-interferon immunoglobulin caused a distinct decrease in the intensity of fluorescence of the interferon-Con A-Sepharose granules. Addition of "pseudo" or L-cell interferons to the labeled antibodies gave no such effect. A decrease in the intensity of fluorescence was observed on the addition of human leukocytic interferon in a dilution of 2^{-8}. This dilution was taken as the titer of this interferon in the direct fluorescence inhibition test. Comparison of the results of titration of the interferon preparations listed above showed that titers determined in the direct fluorescence inhibition test were a little lower than, whereas those of the indirect test were equal to, titers obtained in the virus cytopathic action inhibition test (Table 1).

It was thus shown that, in principle, human leukocytic interferon can be titrated with labeled anti-interferon antibodies. The use of the immunologic fluorescence inhibition test gives reproducible results during titration of different preparations of human leukocytic interferon, and it evidently possesses high sensitivity when used to determine this substance. An important factor is that this test greatly simplifies the process of titrating interferon and makes it much quicker.

In the writers' view, the results can serve as the basis for further studies of the antigenic and species specificity of the various interferons. The possibility cannot be

ruled out that virus, immune, and other types of interferons can be distinguished by means of labeled anti-interferon antibodies. The immunologic fluorescence inhibition test may perhaps make it possible to determine interferon in different tissues and sera. This would be particularly important for, as the writers have shown [12], in allergic and autoimmune diseases increased synthesis of interferon and its accumulation in the blood are observed. The possibility cannot be ruled out that determination of interferon by the immunologic fluorescence inhibition test will become an express method for preliminary assessment of an allergic or autoimmune state.

Labeled anti-interferon antibodies can evidently be used not only for the quantitative determination of interferon. Since interferon participates in immunity and allergy [2, 5], investigations of this mediator on the surface of living cells, especially lymphocytes, can give valuable results in the study of this problem [12]. It will be possible to use the labeled anti-interferon antibodies method to study the dynamics of interferon synthesis in various systems. Furthermore, the writers consider that anti-interferon antibodies can be determined with respect to standard interferon in the immunologic fluorescence inhibition test. Considering the first successful trials of immunologic determination of interferon with the aid of fluorescent antibodies, it can be concluded that other immunologic methods and, in particular, immunoenzymic methods, can be used for the indication of interferon.

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