INVESTIGATION OF THE INTERFERON-INDUCING PROPERTIES OF γ-GLOBULIN AND ITS Fab^1-FRAGMENT

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Human γ-globulin in the aggregated form has the property of inducing interferon formation in mice. The aggregated protein fraction obtained from a preparation of γ-globulin previously heated to 63°C possesses interferon-inducing activity which is 3-4 orders of magnitude higher than the activity of native γ-globulin. The γ-globulin fraction from which all aggregates have been removed no longer possesses the ability to induce interferon. Highly purified preparations of bovine and rabbit γ-globulins induce interferon formation in rabbits, and heterologous γ-globulin is more active than homologous. The pepsin Fab^1-fragment isolated from homologous rabbit γ-globulin has interferon-inducing activity commensurate with that of the unsplit γ-globulin.

Choice of effective interferon-inducing agents is an urgent task in present-day theoretical and applied virology and immunology. Reports of the experimental discovery of interferon-inducing activity in commercial preparations of γ-globulin are of great interest in this connection [4, 5].

The object of this investigation was to study the mechanism of the interferon-inducing activity of γ-globulin. The basic assumption was that it is the aggregated fraction of γ-globulin, formed either spontaneously [18] or after careful heating of the protein preparations [8], which possesses this property. Aggregated γ-globulin can be bound by the tissues and can induce many pathophysiological responses [1, 10, 11] in the same way as bacterial endotoxins [4], the interfon-inducing activity of which has been clearly established [6, 17]. The connection between the species-specificity of γ-globulin and its ability to induce interferon formation were also investigated and the interferon-inducing action of the Fab^1-fragment of the γ-globulin molecule was studied.

EXPERIMENTAL

Commercial preparations of human γ-globulin produced by the Moscow Institute of Epidemiology and Microbiology (batch Nos. 74 and 278) were used. Highly purified preparations of rabbit and bovine γ-globulins were obtained by ion-exchange chromatography on DEAE-Sephadex A-50 as described previously [2, 3]. The 3.5 S pepsin Fab^1-fragment of rabbit γ-globulin was obtained by Nisonoff's method [15] with further purification on CM-cellulose [12].

Fractions of human γ-globulin free from aggregates (FA) and aggregated (A) were obtained by preparative ultracentrifugation as described by Abdou and Richter [8].

To obtain γ-globulin with a high proportion of aggregates, the preparation was incubated for 30 min at 63°C [8] and then centrifuged in the same way as the native preparation in order to obtain A- and FA-fractions.

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TABLE 1. Interferon-Inducing Activity of Human γ-Globulin and Its Fractions (Experiments on Mice)

Type of material tested	Unheated γ-globulin			Heated γ-globulin		
	dilution	interferon titer		dilution	interferon titer	
		after 6 h	after 24 h		after 6 h	after 24 h
Original	10^{-2}	2048	64	10^{-3}	4096	—
	10^{-3}	256	256	10^{-4}	2048	—
	10^{-4}	—	—	10^{-5}	128	—
Before injection	10^{-2}	2048	4096			
	10^{-3}	2048	2048			
	10^{-4}	512	256			
	10^{-5}	—	—	10^{-5}	4096	4096
	10^{-6}	—	—	10^{-6}	256	256
After injection	10^{-2}	128	—	10^{-7}	128	—
	10^{-3}	256	—	10^{-2}	256	—
	10^{-4}	—	—	10^{-3}	—	—
				10^{-4}	—	—

Note: Initial protein concentration in all preparations tested was 5 mg/ml. Interferon titers expressed as reciprocals of dilution; dash indicates that no interferon was found.

TABLE 2. Interferon-Inducing Activity of Heterologous and Homologous γ-Globulin and Fab[1]-Fragment of Homologous γ-Globulin (Experiments on Rabbits)

Time of taking blood samples	Rabbit γ-globulin		Fab[1]-fragment of rabbit γ-globulin		Bovine γ-globulin	
	group 1	group 2	group 1	group 2	group 1	group 2
Before injection	—	—	—	—	—	—
After injection:						
2 h	16	16	32	8	32	32
» 4 »	32	32	32	8		
» 6 »	32	32	32	8	128	128
» 8 »			32	8		
» 12 »	32	64	32	32	256	256
» 24 »	16	16	16	32	32	32
» 30 »	8	8	—		16	16
» 48 »	—	—				—

Note: Protein concentration in all preparations tested was 0.5 mg/ml. Legend as in Table 1.

The interferon-inducing activity of the human γ-globulin preparations was studied by intravenous injection in various dilutions into 8-10 mice weighing 18-20 g. Blood was taken from the heart 6 and 24 h after the injection. The interferon titer was determined in pooled sera collected from 4 to 5 mice and treated by the standard method.

The interferon-inducing activity of preparations of bovine and rabbit γ-globulins and of the Fab[1]-fragment of rabbit γ-globulin were tested on rabbits weighing 2-2.5 kg. The preparations were injected intravenously in a dose of 0.5 mg. Blood was taken both before and 2,6, 12, 24, 30, and 48 h after the injection. To determine the interferon, the mixed sera from two rabbits of each group were treated in the same way as the mouse sera.

Mouse and rabbit interferons were titrated on a transplantable line of L cells and a transplantable line of rabbit embryonic myodermal cells, respectively, by the method based on delay of the cytopathogenic effect of vesicular stomatitis virus.

EXPERIMENTAL RESULTS

The results showing the interferon-inducing activity of native and heated human γ-globulins and its A and FA fractions are given in Table 1. The results of these experiments can be summarized as follows.

1. The fraction of aggregated γ-globulin obtained from the native preparation has significantly higher interferon-inducing activity if compared not only with the aggregate-free fraction but also with the original γ-globulin.

2. After heating of the γ-globulin to 63°C the pattern described above became still more demonstrative. The FA fraction then possessed virtually no interferon-inducing activity, while the activity of the A fraction became 3 or 4 orders of magnitude higher than that of the native γ-globulin.

Clearly, therefore, the aggregated fraction of γ-globulin has the ability to induce interferon formation. The more effectively the aggregated γ-globulin was separated from protein in a monodisperse form, the less interferon-inducing activity was possessed by the FA-fraction.

In the next series of experiments, in order to test their species-specificity the interferon-inducing action of highly purified preparations of rabbit and bovine γ-globulins was investigated in experiments on rabbits. The preparations for comparison did not contain detectable quantities of A-fraction in the native form, as ultracentrifuging showed. This fact evidently explains their low interferon-inducing activity (Table 2). However, heterologous (bovine) γ-globulin induced higher titers of interferon than homologous γ-globulin. It is an interesting fact that the Fab^1-fragment, composing only one-third of the γ-globulin molecule and formed by the light chain and the N-terminal half of the heavy chain [9], is identical in its ability to induce interferon with the unsplit molecule of this protein. After injection of the Fab^1-fragment and native γ-globulins into rabbits, a pyrogenic response and transitory leukopenia, followed by leukocytosis, were observed. The dynamics of these changes corresponded exactly with that described previously [1].

It can be postulated on reasonably safe grounds from the results described in this paper that the interferon-inducing activity is a property of the γ-globulin itself and not of any impurities contained in the preparations (e.g., viruses or polysaccharides). Evidence that this conclusion is correct is given below. As has already been said, the interferon-inducing activity of γ-globulin is determined by its aggregated fraction, and this fraction, if obtained from previously heated γ-globulin, possesses 10,000 times greater activity per unit weight than the native preparation of unheated γ-globulin (Table 1), although it accounts for only 25% of the total quantity of protein in the preparation. In other words, if hypothetical impurities were included in the A fraction, their ratio per unit of protein could have increased by only 4 times, and not by 10,000 times, as follows from the experiment. The results for the low interferon-inducing activity of homologous γ-globulin described in this paper and also mentioned previously [7] as well as data on the interferon-inducing activity of the Fab^1-fragment, to obtain which required additional methods of purification compared with γ-globulin, can serve as additional arguments. It should be specially emphasized that the interferon-inducing action of the Fab^1-fragment, like its other biological properties [1], evidently require the participation of endogenous γ-globulin (homoreactant) [13, 16] with which this fragment interacts in the body.

The results of these experiments suggest that γ-globulin and its Fab^1-fragment may play an essential role as the mediator in the induction of serum interferon by substances of different chemical nature.

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