DISTINCT ANTIGENIC CHARACTER OF TWO COMPONENTS OF POLY(I)·POLY(C)-INDUCED MOUSE L CELL INTERFERON

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1. Introduction

Interferons comprise a heterogeneous group of (glyco)proteins which, in addition to other biological activities, by definition have antiviral properties (reviewed [1]).

Most of our knowledge concerning the heterogeneity of interferon preparations has been obtained from studies of human interferon species. A classification based mostly on its antigenic character is currently being used. So-called human type I interferon, induced by viruses and double-stranded polyribonucleotides, comprises two antigenically distinct interferon species: leukocyte (Le) interferon, produced mainly by leukocytes and lymphoblastoid cell lines and fibroblast (F) interferon, formed mostly by fibroblast cell lines [2–4].

The Le type contains at least two subspecies, differing in size [5]; the F type seems to be homogeneous in this respect [6]. The type of interferon produced seems, besides on the cell type, also to be dependent on the mode of induction. Human fibroblasts incubated with polyinosinic·polycytidylic acid (poly(I)·poly(C)) produce only F interferon, however, after induction with viruses also some Le interferon is formed [7]. Although data in [8] indicate that differences exist between various interferon preparations depending on the producer cell, our knowledge concerning the antigenic character of mouse interferon is limited. Here we present data on the antigenic character of two major components of mouse L cell interferon.

2. Materials and methods

2.1. *Cell culture*

L-929 cells (Flow labs, Irvine, Scotland) were grown in monolayer in Eagle's minimal essential medium (MEM) supplemented with 10% foetal calf serum, non-essential amino acids and gentamicin (80 μg/ml) at 37°C in a 5% CO_2 atmosphere. Culture media were purchased from Flow labs.

2.2. *Interferon production*

Interferon was produced essentially as in [9]. Confluent monolayers of L-929 cells were incubated for 6 h in MEM containing per ml, 20 μg poly(I)·poly(C) (Miles labs, Elkhart, IN) and 0.8 mg diethylaminoethyl (DEAE)–dextran (mol. wt 5×10^5; Pharmacia, Uppsala). Next, cell layers were washed twice with MEM and incubated overnight in MEM. Subsequently, the culture fluid was collected, centrifuged for 15 min at $1200 \times g$ and concentrated by ultrafiltration (PM 10 filter, Amicon, Lexington, MA). Interferon solutions were dialyzed against phosphate-buffered saline (PBS, 0.02 M phosphate (pH 7.2), 0.15 M NaCl), filter sterilized and stored at −70°C.

2.3. *Gel filtration*

Interferon samples (1 ml in PBS) were layered on a Sephadex G-75 superfine column (2.6 × 35 cm, Pharmacia). Elution was carried out in PBS at 12 ml/h at 4°C. Fractions (2 ml) were collected and assayed for interferon activity. Marker proteins (albumin 67 000, ovalbumin 45 000, chymotrypsinogen 25 000 and cytochrome *c* 12 500) were analyzed on the same column under identical experimental conditions.

2.4. *Preparation of anti-interferon sera*

Anti-interferon sera were prepared by monthly injections of New Zealand White rabbits with 10^7 units interferon (spec. act. 10^6 units/mg protein). The first injection was mixed in Freund's incomplete adjuvant (Difco, Detroit, MI), following injections,

starting 2 months after the first one, were without adjuvant. All samples were injected subcutaneously. About 1 week after each injection animals were bled, serum prepared, incubated for 30 min at 56°C and stored at −70°C.

2.5. *Interferon and anti-interferon assays*

Interferon activity was assayed on L-929 cells using vesicular stomatitis virus (VSV) as a challenge by essentially the method in [10]. Titres are expressed in reference units/ml in terms of reference standard G-002-904-511.

For assay of anti-interferon sera, serial dilutions of antiserum were incubated with appropriate amounts of interferon for 1 h at 37°C. Subsequently, samples were analyzed for antiviral activity on L-929 cells using VSV as a challenge as above. The neutralizing titre is expressed as the highest dilution of antiserum which is able to neutralize the antiviral activity of indicated amounts of interferon.

3. Results and discussion

Interferon was produced on L-929 cells, cultured in monolayer, by induction with poly(I)·poly(C) and diethylaminoethyl (DEAE)–dextran. Poly(I)·poly(C)-induced mouse L cell interferon can be separated into at least two different compounds by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) ([11], J. T. unpublished). These species have mol. wt ~38 000 and 22 000, respectively. Besides SDS–PAGE we have also applied gel filtration under mild, non-dissociating conditions for analysis of poly(I)·poly(C) interferon. We have chosen this procedure to be certain that no artificial modifications would be introduced during experi-

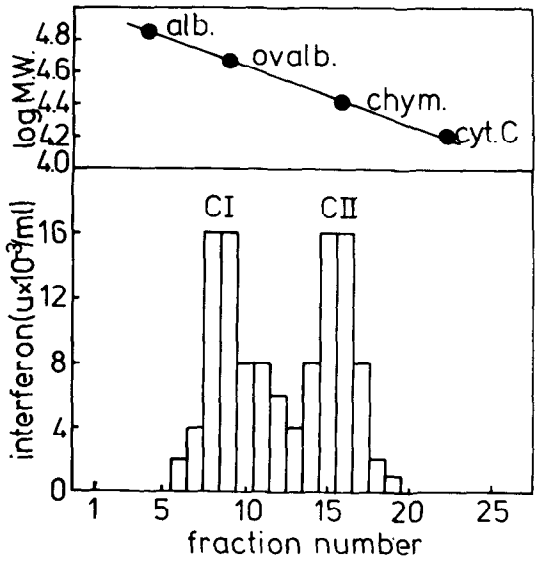

Fig.1. Gel filtration analysis of poly(I)·poly(C)-induced mouse L cell interferon.

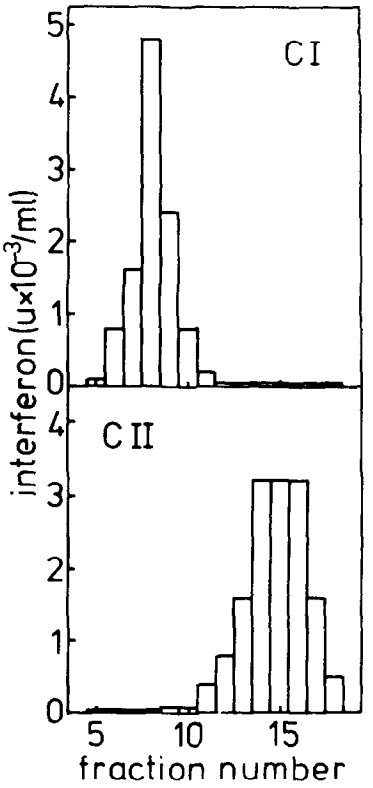

Fig.2. Gel filtration analysis of separated components of poly(I)·poly(C) induced mouse L cell interferon. Pooled fractions of C1 and C2 were analyzed on Sephadex G-75 as in section 2.

Table 1
The effect of anti-interferon sera on the antiviral activity of mouse L cell interferon components

Interferon (units/ml)	Type	Neutralizing titre	
		Serum 5125-4	Serum 5011-2
40	C1	3840	5120
400	C1	480	320
40	C2	5120	<40
400	C2	640	<40

mental handling. Under the conditions applied (PBS) the interferon preparation separates also into two major antiviral components. The apparent molecular weights observed here are somewhat higher than found by SDS–PAGE (48 000 (C1) and 28 000 (C2), respectively) (fig.1). The components are present in ~1:1 molar ratio. The distinct mobility of both components was confirmed by re-analysis of pooled fractions of both molecular species under the same experimental conditions (fig.2). Both components were eluted from the column at the same position as found in the original preparation.

For a further characterization we investigated the effect of different isolates of two antisera raised against the total interferon preparation. The antisera reacted in a completely different way in a neutralization assay with isolated C1 and C2. One of the antisera (5125-4) was able to neutralize both the high and low molecular weight component (table 1). However, an early isolate of antiserum 5011 (5011-2) showed a strong preference to C1 and had no neutralizing activity against C2. Later isolates of this antiserum also neutralize C2 (J. T. in preparation). The data obtained with antiserum 5011-2 implicate that there is a difference in antigenic character between the two molecular species of poly(I)·poly(C)-induced mouse L cell interferon. This is also further evidence that C1 is not a dimer of C2. Several explanations are possible for the finding that C1 and C2 react distinctly with antiserum 5011-2.

(i) It may be that C1 and C2 are completely different molecules.
(ii) It can be that C2 is a double-component of which one component is partially or completely different from C1.
(iii) C1 may be partially identical to C2 but also contain groups which are not present in C2 and that antiserum 5011-2 is specific for these parts of the molecule.

At this moment, based on antigenic studies alone, no firm conclusions can be drawn. Therefore it will be necessary to prepare antibodies against isolated C1 and C2 separately and investigate their cross-reactivity. However, based on other studies, it is tempting to speculate that the third possibility is the correct one.

Recent structural analyses [12,13] indicate that peptide maps and amino acid composition of both components of viral-induced mouse fibroblast interferon are closely related to each other. Furthermore, it has been shown that, concerning viral interferon, the high molecular weight component contains carbohydrate moieties, which are probably absent from the low molecular weight species [14,15]. It might be that besides differences in amino acid composition these differences in carbohydrate content play a role in the observed distinct antigenic properties of both components.

However, it remains to be proven whether interferon synthesized by mouse fibroblasts and induced with poly(I)·poly(C) is identical to viral-induced interferon or whether the same situation exists as in the human system, in which differences have been noticed. So far, both molecular weight analyses ([11], J. T. unpublished) and antigenic studies ([16], J. T. in preparation) show that both interferons are related to each other, but analysis by CH–Sepharose chromatography [11] indicates differences between both preparations. Experiments are in progress to elucidate this problem. Moreover, it remains to be established whether there is any physiological significance for the occurrence of at least two molecular species of mouse fibroblast interferon. The observations described here may have important implications for further interferon investigations. Since it is now possible to characterize mouse interferon components by antigenic properties this can be a tool to identify interferons which have been produced by different cell types or have been separated by various chromatographic techniques. Moreover, specific antibodies can be used for purification of both components.

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