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ANTIGENIC CROSS-REACTION BETWEEN THE α TYPES OF HUMAN AND MOUSE INTERFERON

Y. KAWADE[1], Y. WATANABE[1], Y. YAMAMOTO[1], J. FUJISAWA[1], B.J. DALTON[2] and K. PAUCKER[2]*

[1] *Institute for Virus Research, Kyoto University, Kyoto 606, Japan; and* [2] *Department of Microbiology, The Medical College of Pennsylvania, Philadelphia, PA 19129, U.S.A.*

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Cross-neutralization of the α and β types of human and mouse interferons was tested using antibodies directed against the heterologous types of interferons. The α type of mouse interferon (MuIFN-α) prepared from L cells was found to be completely neutralizable by high-titered antibody against human α-type interferon (HuIFN-α), although the titers were much lower than those obtained in neutralization reactions with the homologous interferon. MuIFN-α from virus-induced lymphocytes, as well as non-glycosylated MuIFN-α from L cells, reacted similarly. The cross-reaction was also observed by the binding of anti-HuIFN-α antibody to a column of immobilized MuIFN-α. The binding experiment indicated that only a small fraction of the anti-HuIFN-α antibody population is heterologously reactive. Reciprocally, HuIFN-α from human leukocytes was neutralized completely by antisera directed against MuIFN from L cells, again with relatively low antibody titers, and the antibody responsible for the heterologous reaction was shown to be the anti-MuIFN-α and not anti-MuIFN-β type. It is concluded that the α types of human and mouse interferon bear an antigenic homology. On the other hand, no significant antigenic cross-reactivity has been detected between the β types of human and mouse interferons.

human interferons mouse interferons antigenicity

INTRODUCTION

Antigenic properties of interferons have played a crucial role in studies on the relationships among various molecular species of interferon, and served to establish the classification of human and mouse interferons into the α, β and γ types [20]. However, they have yielded little information about the relationships of interferons from different animal species. In fact, their antigenicities were found to be largely distinct from each other [13,16,18], in spite of an expectation for the presence of a common or similar structure, based on the similarities of their biological activities. Recently, complete or partial

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determinations of amino acid sequences [1,12,14,23,25,30] have revealed considerable homologies between human and murine α types of interferon (HuIFN-α and MuIFN-α) on the one hand, and between human and murine β types of interferon (HuIFN-β and MuIFN-β) on the other. At present, nothing is known as to how these primary structures are related to the antigenicity. Recent reports indicated that certain subtypes of HuIFN-α and MuIFN-α do cross-react in neutralization reactions with antisera raised against either of them [7,19,21]. We here describe cross-reactivities between HuIFN-α prepared from human leukocytes and MuIFN-α prepared from mouse L cells or lymphocytes. An initial part of this study has been reported preliminary [11,17].

MATERIALS AND METHODS

Mouse interferons

The preparation and partial purification of mouse L-cell interferon have been described [17,29]. The α and β type populations were separated from each other by polyacrylamide gel electrophoresis at pH 4.3 of a preparation with a specific activity of 3×10^7 units/mg protein. Each of the preparations was found to be completely neutralizable by the respective homologous antisera specific for MuIFN-α or -β [28], indicating the absence of contamination by the other type.

Non-glycosylated L-cell interferon was obtained as described earlier [5] by including tunicamycin (2 μg/ml) in the incubation medium of virus-induced cells. The interferon was partially purified by affinity chromatography on a column of immobilized anti-L-cell interferon antibody [9].

For some experiments, MuIFN-α from mouse spleen cells induced by live or UV-inactivated Sendai virus [8] was used. The original interferon preparations contained both MuIFN-α and -β species [27], and the former was isolated by affinity chromatography on an anti-MuIFN-α antibody column [27].

Human interferons

HuIFN-α from human leukocytes was supplied by Dr. K. Cantell (State Serum Institute, Helsinki, Finland) and Dr. H. Saji (Red Cross Blood Center, Kyoto, Japan). HuIFN-β was induced in MG-63 cells by double-stranded RNA, according to Billiau et al. [2], and partially purified by chromatography on SP-Sephadex to a specific activity of $> 1 \times 10^6$ units/mg protein (Watanabe and Kawade, in preparation).

Antisera

The following antisera were used.

1. Antisera against human leukocyte interferon (predominantly anti-HuIFN-α). No. 1 (sheep) was produced as described by Paucker and Dalton [17], and No. 2 (rabbit) was obtained from Dr. J. Vilček (New York University, New York, NY).

2. Antisera against human diploid fibroblast interferon (anti-HuIFN-β). Nos. 1 and 2 (rabbit) were from Dr. J. Vilček, and No. 3 (rabbit) was from Dr. S. Kobayashi (Basic Research Laboratories, Toray Industries, Kamakura, Japan).

3. Antisera against mouse L-cell interferon (mixtures of anti-MuIFN-α and anti-MuIFN-β). No. 1 (sheep) was produced as described by Paucker and Dalton [17], and No. 2 (rabbit) by Yamamoto and Kawade [28].

The homologous neutralization titers of these antisera are given in Tables 2 and 4.

Interferon assay

Interferons were assayed by a cytopathic effect (CPE) reduction method as described previously [17,28]. In most experiments, human interferon was assayed using FS-7 cells and vesicular stomatitis virus (VSV). One experimental unit (E.U.), defined by the 50% CPE reduction point, corresponded to 0.2–1.0 international unit (I.U.) for HuIFN-α, and 1–2 I.U. for HuIFN-β, as calibrated by the NIH human reference interferons G-023-901-527 and G-023-902-527, respectively. L cells and VSV were used for mouse interferon assays. One E.U. ranged from 1.5 to 5.0 I.U., using the NIH mouse reference interferon, G-002-904-511.

Neutralization test

The neutralization of interferon was measured as described previously [17,28]. In most experiments, twofold dilution series of interferon, mixed with a fixed concentration of antibody, were given to the assay cells, and the interferon concentration, i (E.U./ml), that was reduced to 1 E.U./ml of free interferon (that is, 50% CPE) was determined by interpolation. The neutralization titer, $t_{1/10}$, of the serum is defined as the reciprocal of the serum dilution that reduces the interferon concentration to one-tenth, and calculated by the formula, $t_{1/10} = s\ (i - 1)/9$, where s denotes the serum dilution [10] (the antibody and interferon concentrations are the final dilutions in the culture fluid of the assay cells). The titers were calculated using s values which gave i values in a range between 4 and 40 E.U./ml.

Fractionation of antibodies by immobilized interferon

Ten million I.U. of electrophoretically homogeneous L-cell α- and β-types of interferons [28], with specific activities of 2.5×10^7 and 1×10^8 I.U./mg protein, respectively, were coupled separately to CNBr-Sepharose (2 ml bed volume) [27]. Antiserum was loaded to the column, which was washed with Dulbecco's phosphate-buffered saline minus Ca^{2+} and Mg^{2+} (PBS), until the UV absorbance fell to the background level ('flow-through' fraction). The adsorbed antibody was then eluted by means of 0.5 M acetic acid containing 0.5 M NaCl ('retained' fraction). When fractionating high-titered anti-L-cell interferon antibody, 0.01 N HCl was used for elution instead of the acetic acid,

yielding higher recoveries (control experiments had indicated that both the anti-MuIFN-α and anti-MuIFN-β activities of this serum were completely stable upon exposure to these eluants). The fractions were concentrated by pressure dialysis against PBS using collodion membranes (Sartorius SM 132 00).

RESULTS

Neutralization of mouse interferon by anti-human interferon sera

MuIFN-α and -β from L cells were tested, employing L cells, for the neutralization against anti-HuIFN-α and anti-HuIFN-β antisera. As a sensitive test for the neutralization reaction, the interferon dose–response curve in the presence of antiserum (at a fixed concentration) was determined and compared with the control curve without added antiserum. As illustrated in Fig. 1, the interferon dose–response curve for MuIFN-α was shifted to the right (higher interferon doses) in the presence of anti-HuIFN-α antiserum, and the extent of the shift increased with an increase in the antiserum concentration, indicating a positive neutralization reaction. The values of the neutralization titer were calculated as described in Materials and Methods, and found to be independent of the antiserum concentration, as listed in Table 1. When examined in a similar manner,

TABLE 1

Neutralization of MuIFN-α by anti-HuIFN-α antisera

Antiserum	Expt. No.	Serum dilution tested	Neutralization values[a] obtained in reactions with[b]					
			MuIFN-α(L)		MuIFN-α_0(L)		MuIFN-α(Lym)	
			i	$t_{1/10}$	i	$t_{1/10}$	i	$t_{1/10}$
Anti-HuIFN-α, No. 1	1	1/500	42	2300	14.5	750		
		1/2000	12	2400	8	1500		
	2	1/1500	37	6000				
		1/6000	9	5300				
Anti-HuIFN-α, No. 2	3	1/50	25	130	21	110		
		1/200	5.3	100	7	130		
	4	1/100	13	130	6.5	60	11	110
		1/400	3.7	120	3.5	110	5.6	200

[a] The interferon concentration i (E.U./ml) that was reduced to 1 E.U./ml by the indicated concentration of antiserum was determined, and the antibody titer $t_{1/10}$ was calculated as described under Materials and Methods.

[b] MuIFN-α(L): α-type interferon from L cells; MuIFN-α_0(L): non-glycosylated interferon produced by tunicamycin-treated L cells; MuIFN-α(Lym), α-type interferon isolated from Sendai virus-induced mouse lymphocyte interferon by affinity to immobilized anti-MuIFN-α antibody. A similar preparation from lymphocyte interferon induced by UV-irradiated Sendai virus gave similar results.

no significant neutralizations were observed with the other combinations, that is, MuIFN-α with anti-HuIFN-β, and MuIFN-β with anti-HuIFN-α or anti-HuIFN-β. The results, expressed as the neutralization titers, are summarized in Table 2. Three other anti-HuIFN-α antisera (results not shown) with homologous titers similar to or lower than that of antiserum No. 2 showed a weakly positive to insignificant neutralization against MuIFN-α. This indicates that anti-HuIFN-α antibodies are heterogeneous in their reactivity to MuIFN-α, most of them being non-reactive (see below).

Non-glycosylated interferon molecules are produced by L cells in the presence of tunicamycin [5], which were previously shown to consist solely of the MuIFN-α antigenic type [6]. This type of interferon was also found to be neutralized by the anti-HuIFN-α antiserum Nos. 1 and 2, and not by anti-HuIFN-β. As indicated in Table 1, the neutralization titers of the two anti-HuIFN-α antisera were similar to those against the glycosylated MuIFN-α.

Mouse interferons produced by virus-induced lymphocytes consist of MuIFN-α and -β type antigenic molecules [27]. The MuIFN-α species were isolated by affinity chromatography using immobilized anti-MuIFN-α antibody. They were also neutralized by anti-HuIFN-α antisera in the same manner as described for MuIFN-α isolated from L cells, as shown in Table 1.

Binding of anti-HuIFN-α antibody to mouse interferon immobilized on Sepharose

The reaction between MuIFN-α and anti-HuIFN-α antibody was then verified in an independent way, by binding of the antibody to purified interferon immobilized on

TABLE 2

Neutralization of human and mouse interferons by anti-human interferon sera

Antiserum	Neutralization values[a] obtained in reactions with[b]				
	Human interferon		Mouse interferon		
	HuIFN-α	HuIFN-β	Unfractionated	MuIFN-α	MuIFN-β
Anti-HuIFN-α, No. 1	1,500,000	900	< 45	4,000[d] (1,800–6,000)	< 45
Anti-HuIFN-α, No. 2	4,000	< 100	ND[c]	150[d] (100–270)	< 10
Anti-HuIFN-β, No. 1	< 100	5,000	ND	< 10	< 10
Anti-HuIFN-β, No. 2	< 100	2,000	ND	< 10	< 10
Anti-HuIFN-β, No. 3	< 100	5,000	ND	< 10	< 10

[a] $t_{1/10}$ values, determined as described in Materials and Methods.

[b] HuIFN-α, from human leukocytes; HuIFN-β from MG-63 cells: mouse interferons from L cells.

[c] Not done.

[d] Geometric mean and range of values obtained in several determinations.

Sepharose. Anti-HuIFN-α antiserum (No. 1) was loaded on MuIFN-α- and MuIFN-β-containing columns and fractionated into the flowthrough and retained fractions (the latter eluted with acid). The fractions were assayed for neutralization of MuIFN-α and HuIFN-α. When fractionated on the MuIFN-α column, all the MuIFN-α-neutralizing activity in the original antiserum was found in the retained fraction, and virtually none in the flowthrough fraction, as shown in Table 3. The assays of the same fractions against HuIFN-α indicated that most of the applied antibodies appeared in the flowthrough fraction, while only 10% was in the retained fraction (Table 3). Upon rechromatography of the retained fraction on the same MuIFN-α column, both the HuIFN-α- and MuIFN-α-neutralizing activities were quantitatively recovered in the retained fraction (not shown), indicating that specific fractionation of the antibodies had occurred.

On the other hand, the same anti-HuIFN-α antiserum loaded on the MuIFN-β column showed very little retention of the antibody (Table 3), in harmony with the lack of neutralization in this combination (MuIFN-β vs. anti-HuIFN-α).

Neutralization of human interferon with anti-mouse interferon sera

Cross-neutralization of HuIFN-α with anti-L-cell interferon antibody, as noted previously [17], was confirmed with two batches of antisera, although the titers were again low compared to the homologous titers, as shown in Table 4. HuIFN-β showed no sign of neutralization with these antisera.

Since these antisera contain both anti-MuIFN-α and anti-MuIFN-β antibodies (Table 4), it would be desirable to determine which of these is causing the heterologous neutralization reaction. Unfortunately, the monospecific antisera obtained by immun-

TABLE 3

Fractionation of anti-HuIFN-α antibodies by affinity to immobilized mouse interferons[a]

Antibody fraction	Neutralization units[b] against	
	HuIFN-α	MuIFN-α
Original serum	380,000	400
MuIFN-α column		
flowthrough	230,000	10
retained	37,000	1,000
MuIFN-β column		
flowthrough	310,000	200
retained	800	20

[a] Anti-HuIFN-α antiserum (No. 1, 0.1 ml) was fractionated on columns of immobilized MuIFN-α or MuIFN-β into flowthrough and retained fractions, as described in Materials and Methods, and their neutralization titers against HuIFN-α and MuIFN-α were determined.

[b] Neutralization titer × volume of the fraction in ml.

TABLE 4

Neutralization of human and mouse interferons by anti-L-cell interferon sera

Antiserum	Neutralization values[a] obtained in reactions with[b]				
	Human interferon		Mouse interferon		
	HuIFN-α	HuIFN-β	Unfractionated	MuIFN-α	MuIFN-β
Anti-L IFN, No. 1	2,400[d] (1,100–7,000)	< 20	220,000	350,000	200,000
Anti-L IFN, No. 2	40[d] (30–160)	ND[c]	1,300	23,000	800

[a–d] See footnotes to Table 2.

ization with isolated MuIFN-α and -β [28] are of relatively low homologous titers (1000 and 3000, respectively, recalculated according to Kawade [10]), and showed little heterologous neutralization. This problem was approached by fractionating the high-titered anti-L-cell interferon serum (No. 1) on columns of immobilized MuIFN-α and -β, respectively. The result is shown in Fig. 2. The original antiserum had neutralizing activities against the three interferons, MuIFN-α, MuIFN-β and HuIFN-α, as shown in

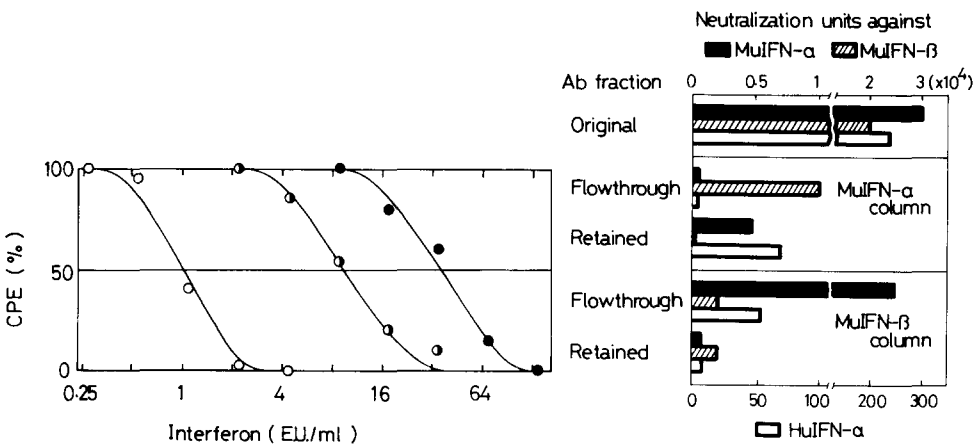

Fig. 1. Neutralization of MuIFN-α from L cells by anti-HuIFN-α antiserum (No. 1). The interferon dose–response curves were determined in L-cell cultures in the absence (○) and presence of the antiserum at dilutions of 1 : 6000 (◑) and 1 : 1500 (●). One E.U. was equal to 2.6 I.U. in this assay. Neutralization titers calculated from this data are listed in Table 1, Expt. 2.

Fig. 2. Fractionation of anti-L-cell interferon antiserum (No. 1) by columns containing immobilized mouse α- and β-type interferons. One-tenth ml of the antiserum was fractionated by each column, and the fractions were assayed against MuIFN-α, MuIFN-β, and HuIFN-α. Neutralization units (neutralization titer × volume of the fraction in ml) are indicated.

the top panel of Fig. 2. When fractionated on the MuIFN-α column (middle panel), the flowthrough fraction, as expected, was found to consist largely of anti-MuIFN-β, and to contain little anti-MuIFN-α activity. Also, it showed little heterologous neutralizing activity against HuIFN-α. The retained antibodies were difficult to elute, and only 16% of the applied anti-MuIFN-α activity could be recovered by acid elution. This fraction, in contrast to the flowthrough, contained the heterologous neutralizing activity against HuIFN-α.

The other column containing MuIFN-β (lower panel) also effectively absorbed the homologous antibody, resulting in the flowthrough fraction greatly enriched in anti-MuIFN-α. This fraction showed a significant anti-HuIFN-α activity. Elution of the retained antibody by acid recovered only 10% of the applied anti-MuIFN-β activity. The specificity of the retained fraction was unexpectedly poor, having considerable activities against MuIFN-α and HuIFN-α as compared to those against MuIFN-β. The reason for this is not clear.

These results thus indicated that, upon fractionation of the anti-mouse interferon antibody mixture by means of affinity to immobilized MuIFN-α and -β, the heterologous neutralization activity against HuIFN-α accompanied anti-MuIFN-α but not anti-MuIFN-β.

DISCUSSION

The experiments described in this study show that HuIFN-α and MuIFN-α are cross-neutralizable by the respective heterologous antibodies, although the heterologous titers were much lower than the homologous ones and potent antisera were needed to show the neutralization. The cross-reaction was also shown by the binding of anti-HuIFN-α antibody to MuIFN-α immobilized on Sepharose. The antigenic homology observed here confirms the similarity noted earlier between HuIFN-α and the 24K interferon component from mouse L-cells (MuIFN-α) [11]. Although similarities were also shown to exist between HuIFN-β and the 36K interferon species (MuIFN-β) from mouse L cells [11], we have so far been unable to detect any appreciable antigenic cross-reactivity. One possible explanation for this may be that the anti-HuIFN-β antisera had relatively low homologous titers. This, however, does not apply to the anti-L-cell interferon serum (No. 1), which had a high homologous titer against MuIFN-β comparable to that against MuIFN-α.

N-Terminal amino acid sequences of some types of human and mouse interferon have recently been reported. The α-type interferon from Ehrlich ascites tumor cells [3], previously called the C component [23], has a marked homology to HuIFN-α, that is, 13 amino acids out of 20 for Namalva cell interferon [30], and 10 and 11 out of 20 for HuIFN-α_1 and -α_2, respectively, from human leukocytes [14,22]. This is in harmony with the antigenic homology found in this study between HuIFN-α and MuIFN-α, although it is not known whether the N-terminals are the antigenic sites. On the other hand, the N-terminal sequences of the β types of interferon (called A, 35–40K, and B, 26–33K) of Ehrlich ascites tumor cell interferon [23] also have a considerable homology

with that of HuIFN-β from diploid fibroblasts, deduced from the base sequence of the cDNA [4,25], that is, eight amino acids out of 24, and yet no significant antigenic homology has been found between HuIFN-β and MuIFN-β. Further studies are needed to clarify the relationship between the primary structure and the antigenic properties. It may be noted in this connection that HuIFN-α and -β have a considerable sequence homology [24], but no antigenic cross-reactions have so far been reported.

The type of neutralization test used in this study (illustrated in Fig. 1) is a sensitive one, because it can detect the shift in the interferon titration endpoint at the level of 1 unit/ml. It is especially suited for examining interferon preparations containing both neutralizable and non-neutralizable species. Thus, when an interferon preparation is completely neutralizable by a given antiserum, the shift of the interferon dose–response curve toward the right, caused by the added antiserum, will become simply greater, when higher and higher antiserum concentrations are added. If, on the other hand, there is a non-neutralizable component, the interferon dose–response curve will not be shifted beyond a certain point, which is determined by the content of the non-neutralizable component, even when large excesses of antiserum are added. Such a neutralization pattern was in fact observed with some anti-HuIFN-α antisera against MuIFN-α (results not shown). This may be interpreted to mean that MuIFN-α is a mixture of subspecies, some reacting, and others not, with the heterologous antibody (see below). On the other hand, the two anti-HuIFN-α antisera described in this paper (as well as the anti-L-cell interferon antisera) showed no such limit in the shift of the interferon dose–response curve with an increase in antibody concentration (Fig. 1 and Table 1), indicating that certain batches of antisera can completely neutralize the heterologous α interferons.

The results on the various batches of anti-HuIFN-α antisera indicated that the heterologous titers against MuIFN-α were not directly proportional to the homologous ones (Table 2 and unpublished results), suggesting that the antibodies against HuIFN-α are not uniform in the heterologous reactivity. This was verified directly by the antibody-binding experiments to immobilized MuIFN-α. The result (Table 3) showed that only a small fraction of the antibody population in the anti-HuIFN-α antiserum was reactive against MuIFN-α.

In examining the antigenicity of mouse interferons induced by virus or polynucleotides, it must be borne in mind that various mouse interferons of fibroblastoid and lymphoid origins contain both MuIFN-α and -β [3,11,26–28]. This is in contrast to the commonly studied human interferons from virus-induced leukocytes and polynucleotide-induced fibroblasts, which essentially consist of single types of interferon, HuIFN-α and -β, respectively. Therefore, when the unfractionated mouse interferon is examined for neutralization by anti-HuIFN-α antisera, the neutralization of MuIFN-α that occurs will not readily be detected [17], because MuIFN-β present together remains unneutralized and hides the reaction of MuIFN-α. On the other hand, neutralization of human leukocyte-derived interferon by anti-L-cell interferon serum can readily be observed when high-titered antisera are used [17], because it is practically free of the β-type interferon.

The non-glycosylated L-cell interferon produced in the presence of tunicamycin was

earlier shown to contain only the MuIFN-α antigenic molecules [6], and it was suggested that MuIFN-β, in contrast to MuIFN-α, requires its sugar moiety for the stability or for the synthesis and secretion from the cell [6]. The non-glycosylated MuIFN-α was found here to be similar to the glycosylated form in the reactivity to anti-HuIFN-α (Table 1) as well as to anti-MuIFN-α [6], indicating that the sugar moiety of this species is not important for the antigenicity. A conclusion to the contrary might be obtained if one were to compare the glycosylated preparation with the non-glycosylated one without knowing their α/β compositions, because the non-glycosylated interferon is completely neutralizable by anti-HuIFN-α, whereas the glycosylated one is not (due to the presence of MuIFN-β), giving an impression that deprivation of the sugar moiety causes a change in antigenicity and gives rise to a reactivity to anti-HuIFN-α. Actually, however, the reason why the heterologous neutralization becomes readily demonstrable upon the sugar deprivation is that the β-type interferon is not produced in the presence of tunicamycin [6].

Antigenic cross-reactions between certain human and mouse interferons were recently reported. Thus, a minor 21K component in mouse tissue culture interferons was found to react with anti-HuIFN-α antibody [7,21], and HuIFN-α spontaneously produced by lymphoblastoid cells (but not by virus-induced ones) were neutralized by anti-mouse interferon antiserum [19]. HuIFN-α was recently recognized to comprise various subtypes with slightly different primary structures [1,15], and therefore these findings appear to indicate that certain subtypes of human and mouse interferons may have strong antigenic homologies. Our findings extend this concept in demonstrating that the whole populations of HuIFN-α and MuIFN-α are cross-reactive, though with low neutralization titers.

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REFERENCES

1 Allen, G. and Fantes, K.H. (1980) A family of structural genes for human lymphoblastoid (leukocyte-type) interferon. Nature 287, 408.

2 Billiau, A., Edy, V.G., Heremans, H., Van Damme, J., Desmyter, J., Georgiades, J.A. and De Somer, P. (1977) Human interferon: mass production in a newly established cell line, MG-63. Antimicrob. Agents Chemother. 12, 11.

3 Broeze, R.J., Dougherty, J.P., Pichon, J., Jayaram, B.M. and Lengyel, P. (1981) Studies with pure mouse Ehrlich ascites tumor interferons α and β: patterns of induction of $(2'-5')(A)_n$ synthetase and of a double-stranded RNA-dependent protein kinase in mouse cells and human cells. J. Interferon Res. 1, 191.

4 Derynck, R., Content, J., DeClercq, E., Volckaert, G., Tavernier, J., Devos, R. and Fiers, W. (1980) Expression of human fibroblast interferon gene in *Escherichia coli*. Nature 287, 193.
5 Fujisawa, J., Iwakura, Y. and Kawade, Y. (1978) Nonglycosylated mouse L cell interferon produced by the action of tunicamycin. J. Biol. Chem. 253, 8677.
6 Fujisawa, J. and Kawade, Y. (1981) Properties of nonglycosylated and glycosidase-treated mouse L cell interferon species. Virology (in press).
7 Havell, E.A. (1979) Isolation of a subspecies of murine interferon antigenically related to human leukocyte interferon. Virology 92, 324.
8 Ito, Y., Nishiyama, Y., Shimikata, K., Nagata, I., Takeyama, H. and Kunii, A. (1978) The mechanism of interferon induction in mouse spleen cells stimulated with HVJ. Virology 88, 128.
9 Iwakura, Y., Yonehara, S. and Kawade, Y. (1978) Purification of mouse L cell interferon. Essentially pure preparations with associated cell growth inhibitory activity. J. Biol. Chem. 253, 5074.
10 Kawade, Y. (1980) An analysis of neutralization reaction of interferon by antibody: a proposal on the expression of neutralization titer. J. Interferon Res. 1, 61.
11 Kawade, Y., Yamamoto, Y., Fujisawa, J. and Watanabe, Y. (1980) Antigenic relationships among various interferon species. Ann. N.Y. Acad. Sci. 350, 422.
12 Knight, E., Jr., Hunkapiller, M.W., Korant, B.D., Hardy, R.W.F. and Hood, L.E. (1980) Human fibroblast interferon: amino acid analysis and amino terminal amino acid sequence. Science 207, 525.
13 Levy-Koenig, R.E., Golgher, R.R. and Paucker, K. (1970) Immunology of interferons. II. Heterospecific activities of human interferons and their neutralization by antibody. J. Immunol. 104, 791.
14 Mantei, N., Schwartzstein, M., Streuli, M., Panem, S., Nagata, S. and Weissman, C. (1980) The nucleotide sequence of a cloned human leukocyte interferon cDNA. Gene 10, 1.
15 Nagata, S., Mantei, N. and Weissmann, C. (1980) Structure of one of eight or more distinct chromosomal genes for human interferon-α. Nature 287, 401.
16 Paucker, K. (1977) Antigenic properties. Tex. Rep. Biol. Med. 35, 23.
17 Paucker, K. and Dalton, B. (1980) Antigenic properties of crossprotecting factors in human leukocyte and mouse L cell interferons. Ann. N.Y. Acad. Sci. 350, 332.
18 Paucker, K., Dalton, B.J., Ogburn, C.A. and Törmä, E. (1975) Multiple active sites on human interferons. Proc. Natl. Acad. Sci. U.S.A. 72, 4587.
19 Pickering, L.A., Kronenberg, L.H. and Stewart II, W.E. (1980) Spontaneous production of human interferon. Proc. Natl. Acad. Sci. U.S.A. 77, 5938.
20 Stewart II, W.E. et al. (1980) Interferon nomenclature. Nature 286, 110.
21 Stewart II, W.E. and Havell, E.A. (1980) Characterization of a subspecies of mouse interferon cross-reactive on human cells and antigenically related to human leukocyte interferon. Virology 101, 315.
22 Streuli, M., Nagata, S. and Weissman, C. (1980) At least three human type α interferons: structure of α2. Science 209, 1343.
23 Taira, H., Broeze, R.J., Jayaram, B.M., Lengyel, P., Hunkapiller, M.W. and Hood, L.E. (1980) Mouse interferons: amino terminal amino acid sequences of various species. Science 207, 528.
24 Taniguchi, T., Mantei, N., Schwartzstein, M., Nagata, S., Muramatsu, M. and Weissmann, C. (1980) Human leukocyte and fibroblast interferons are structurally related. Nature 285, 547.
25 Taniguchi, T., Ohno, S., Fujii-Kuriyama, Y. and Muramatsu, M. (1980) The nucleotide sequence of human fibroblast interferon cDNA. Gene 10, 11.
26 Trapman, J. (1980) Distinct antigenic character of two components of poly(I)·poly(C)-induced mouse L cell interferons. FEBS Lett. 109, 137.
27 Yamamoto, Y. (1981) Antigenicity of mouse interferon: two distinct molecular species common to interferons from various sources. Virology 111 (in press).
28 Yamamoto, Y. and Kawade, Y. (1980) Antigenicity of mouse interferons: distinct antigenicity of

the two L cell interferon species. Virology 103, 80.

29 Yamamoto, Y., Tsukui, K., Ohwaki, M. and Kawade, Y. (1974) Electrophoretic characterization of purified mouse L cell interferon of high specific activity. J. Gen. Virol. 23, 23.

30 Zoon, K.C., Smith, M.E., Bridgen, P.J., Anfinsen, C.H., Hunkapiller, M.W. and Hood, L.E. (1980) Amino terminal sequence of the major component of human lymphoblastoid interferon. Science 207, 527.