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ANTIGENIC CORRELATIONS BETWEEN COMPONENTS OF C243 AND L CELL INTERFERONS

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The three components of virus-induced interferon (IFN) from C243 cells, with molecular weights of 19,000, 24,000 and 30,000, were examined for neutralization by antisera against the α (24,000) and β (36,000) types of virus-induced L cell IFN. The results indicated that the C243 19,000 component belongs to the α type, and the 24,000 and 30,000 components to the β type. The 19,000 component was also indistinguishable from L cell IFN-α in the reaction with antisera against human IFN-α.

mouse interferon antigenicity

Mouse interferons (IFN) induced by virus or double-stranded RNA in commonly used cell lines contain two or three components differing in molecular weight: L-cell, 24,000 and 36,000 [9]; C243 cell, 22,000 and 35,000 [3], or 19,000, 24000 and approximately 30,000 [1]; and Ehrlich ascites tumor (EAT) cell, 20,000 and 35,000–40,000 [2] or 20,000, 26,000 and 35,000 [8]. According to the new nomenclature [7], the L 24,000 and the EAT 20,000 components were designated as murine IFN-α, and the L 36,000 and the EAT 26,000 and 35,000 components as murine IFN-β. These designations were based, on the one hand, on the homologies in the N-terminal sequences of the three EAT IFN components to human IFN-α and -β [8], and, on the other hand, on the antigenic homology of the L 24,000 component to human IFN-α [5,6]. The antigenic correlations of L and EAT IFNs have not been directly demonstrated. The C243 IFN components have molecular sizes similar to those of EAT IFNs. However, this evidence by itself is not sufficient for unambiguous identification of their antigenic types. We therefore examined the antigenicity of the three IFN components of C243 cells by neutralization tests with two antisera against the individual components of L cell IFN: L-α (24,000) and L-β (36,000) [11].

The three IFN components of C243 cells were prepared, purified, and electrophoretically separated from each other as described previously [1,9]. IFN was titrated by the cytopathic effect (CPE) reduction method using L cell (line L0) and vesicular stomatitis virus [11]. One unit in this system was equivalent to 1.5–5 international reference units

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in different experiments. Antibody neutralization tests were performed, as described before [4], by determining the IFN dose vs. CPE response curves in the presence of fixed concentrations of antibody, and comparing the IFN titration end-points (50% CPE) with that determined in the absence of antibody. The neutralization titer ($t_{1/10}$) of an antiserum is defined as the reciprocal of the serum dilution that reduces the IFN activity to one-tenth [4]. The antisera used here have been described [5,11].

The results of neutralization tests were clear-cut and indicated that the 19,000 component of C243 IFN belongs to the α type, and the 24,000 and 30,000 components to the β type. As shown in Table 1, the 19,000 component of C243 IFN was efficiently neutralized by anti-L-α antibody, but its IFN titer was not significantly affected by anti-L-β antibody. On the other hand, the 24,000 and 30,000 components of C243 IFN were well neutralized by anti-L-β antibody, but not by anti-L-α antibody. The neutralization titers of these antisera were similar for the corresponding IFN species from the two cell sources.

IFN-α from L cells was previously reported to be completely neutralizable by antisera against human IFN-α (HuIFN-α), though with much lower neutralization titers than the homologous titers [5]. The 19,000 component of C243 IFN was also neutralized by the two anti-HuIFN-α antisera described previously [5] (Table 2), but, like L-α IFN, was not affected at all by high concentrations of antisera against human IFN-β (HuIFN-β). With the 24,000 and 30,000 components of C243 IFN, no significant changes in IFN titer were observed in the presence of high concentrations of either anti-HuIFN-α or anti-HuIFN-β antisera, as in the case of L-β IFN [5].

The heterologous reactivity of anti-HuIFN-α antiserum was shown to be carried by a small fraction of the antibody population, which could be separated from the bulk of antibody by affinity binding to L-α IFN immobilized on Sepharose [5]. These antibody fractions (one retained by the L-α IFN column, and the other, unretained) were tested against the 19,000 component of C243 IFN, to see whether the same small fraction of antibody was responsible for the reaction. As shown in Table 2, practically all the reactivity of the anti-HuIFN-α antiserum against both the C243 19,000 and L-α IFNs was found in the 'retained' antibody fraction, which represented about 1% of the total activity of the original antiserum against HuIFN-α. The bulk of the antibody, recovered in the 'unretained' fraction, was nearly devoid of heterologous reactivity. Thus, in this respect also, the 19,000 component of C243 IFN behaved as L-α IFN.

We conclude that the 19,000 component of C243 IFN and L-α IFN, on the one hand, and the 24,000 and 30,000 components of C243 IFN and L-β IFN, on the other, are antigenically closely related if not identical.

Finally, an antiserum against the β component of IFN from EAT cells (prepared by Dr. O. Yoshie in Dr. P. Lengyel's laboratory at Yale University and kindly provided by Dr. N. Ishida of Tohoku University) was found to neutralize L-β ($t_{1/10} \sim 10{,}000$) but not L-α IFN ($t_{1/10} < 100$), thereby directly verifying the α and β designations of these IFN components.

TABLE 1

Neutralization of individual components of C243 and L cell IFNs by antisera against L-cell α and β IFNs

Antiserum		Neutralization values[a] obtained in reactions with:									
		C243 IFN						L IFN			
		19,000		24,000		30,000		α (24,000)		β (36,000)	
Specificity	Dilution	E	$t_{1/10}$	E	$t_{1/10}$	E	$t_{1/10}$	E	$t_{1/10}$	E	$t_{1/10}$
None		22,000		35,000		18,000		1,200		2,500	
Anti-L-α	1 : 2,000	2,000	2,200	ND[b]		ND		350	530	ND	
	1 : 500	540	2,100	ND		ND		23	2,800	ND	
	1 : 125	ND		30,000	<10	13,000	<10	ND		1,800	<10
Anti-L-β	1 : 3,200	ND		6,500	1,600	6,000	700	ND		210	4,000
	1 : 800	13,000	<100	500	5,600	700	2,200	1,000	<100	25	8,800

[a] E indicates the reciprocal of IFN titration end-point, determined in the presence or absence of antiserum at the indicated dilution; $t_{1/10}$ is the antibody titer defined as the reciprocal of serum dilution that reduces the IFN activity to one-tenth [4].

[b] Not done.

TABLE 2

Neutralization of the 19,000 component of C243 IFN and of L-α IFN by anti-HuIFN-α antibodies

Antibody[c]	Neutralization values[a] obtained in reactions with:						
	C243-19,000			L-α			HuIFN-α[b]
	s	*E*	$t_{1/10}$	*s*	*E*	$t_{1/10}$	$t_{1/10}$
None		6,000			2,300		
Anti-HuIFN-α, No. 2	1,200	2,100	230			100–270[d]	4,000[d]
	120	600	120				
Anti-HuIFN-α, No. 1	2,000	400	3,000	4,000	340	2,500	2,000,000
	400	<50	>5,000	800	80	2,500	
No. 1, L-α-retained	2,000	550	2,200	10,000	600	3,300	15,000
	400	<50	>5,000	2,000	150	3,100	
No. 1, L-α-unretained	2,000	4,000	<200	2,000	1,600	<200	2,000,000
	400	1,000	220	500	800	100	

[a] *s* denotes the reciprocal of antiserum dilution tested; *E* and $t_{1/10}$ are as in Table 1.

[b] Human IFN-α from leukocytes was assayed on human fibroblasts trisomic for chromosome 21 (XT-2101, kindly supplied by Dr. Tetsuo Sudo of Basic Research Laboratories, Toray Industries) using encephalomyocarditis virus as challenge virus.

[c] The antisera Nos. 1 and 2, and the antibody fractions are those described previously [5].

[d] Data from Kawade et al. [5].

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