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LANTHANIDE ION ENHANCEMENT OF INTERFERON BINDING TO CELLS

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SUMMARY: Pretreatment of purified [^{125}I]-labeled human and mouse β interferons (IFN) with lanthanum chloride ($LaCl_3$) enhanced 20-30 fold the binding of the [^{125}I]-IFNs to human A549 and mouse L cells at 0°C and also enhanced antiviral activity in homologous cells. Although lanthanides enhanced cross-species binding of both human and mouse [^{125}I]-IFNs, there was no increase in cross-species antiviral activity. Unlabeled IFN not treated with $LaCl_3$ did not compete with [^{125}I]-IFN treated with $LaCl_3$ for cellular receptors. However, unlabeled IFN treated with $LaCl_3$ did compete with $LaCl_3$-treated [^{125}I]-IFN. These results suggest that lanthanide treated IFNs do not bind to the same receptors as native IFNs.

We have previously reported that the salts of the rare earth elements (lanthanides), in addition to protecting human interferon-β (HuIFN-β) from thermal inactivation, enhance by a factor of two or more the antiviral activity of human α, β, and γ and mouse α/β IFNs (1). To determine if the enhanced antiviral activity of the human and mouse β IFNs in the presence of lanthanide salts is a result of enhanced IFN binding to cells, we have examined the effects of $LaCl_3$ and $YbCl_3$ on the binding of [^{125}I]-IFN-βs of human and mouse origin to homologous and heterologous cells. We observe a 20-30 fold increase in the [^{125}I]-IFN-β bound to homologous cells in the presence of $LaCl_3$ and $YbCl_3$ but this lanthanide-treated IFN does not appear to be bound to high affinity IFN receptors.

MATERIALS AND METHODS

Mouse interferon-β (MuIFN-β) (2.8×10^6 IU/ml, specific activity of 1.3×10^8 IU/mg protein from Lee Biomolecular Research Laboratories, Inc., San Diego, CA) was labeled with the Bolton-Hunter reagent as previously described (2) and had a specific activity of 4.5 μCi/μg. Iodination did not

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reduce the antiviral activity. Antiviral activity of mouse IFN was determined by inhibition of the yield of GDVII virus hemagglutinin in mouse L cells (3). This assay measures 4.13 ± 0.28 $\log_{10}$ units for the NIH mouse L cell IFN standard G002-904-511 (assigned value 4.08). All IFN units are reported as international units (IU) per ml.

Human IFN-β was obtained from Rentschler Arzneimittel GmbH and Co. (Laupheim, West Germany) as a lyophilized preparation. When reconstituted, the preparation contained 10^6 IU/ml and 3.4 mg/ml protein. HuIFN-β was further purified by chromatography on a controlled pore glass (Electro-Nucleonics, Inc., CPG 00350 200/400 mesh, Fairfield, NJ) column (0.4 x 4.1 cm) by the method of Edy et al. (4). The specific activity of the HuIFN-β eluted with 0.1 M KCl-HCl, pH 2.0 was 7.8 x 10^8 IU/mg protein. One μg IFN was reacted with 300 μCi Bolton-Hunter reagent and labeled to a specific activity of 12 μCi/μg (2). The [^{125}I] HuIFN-β showed only one band at apparent molecular weight 16,500. There was no loss of antiviral activity following labeling and purification. Human IFN-β antiviral activity was determined by inhibition of encephalomyocarditis virus hemagglutinin yield in the A549 human lung carcinoma cell line (5). This assay measures a geometric mean of 3.93 ± 0.19 $\log_{10}$ units for the NIH human fibroblast IFN standard G023-902-527, which has an assigned value of 4.0 $\log_{10}$ units. All IFN titers are reported in IU per milliliter. Protein was determined by the Coomassie brilliant blue micromethod (6) with bovine albumin as a standard.

Mouse L cells were grown in suspension cultures as previously described (7). The suspension grown mouse L cells used in binding studies were washed in Tris-Saline and resuspended to 10^8 cells/ml in the same buffer. Human A549 cells were grown in Falcon plastic flasks as previously described (8). The A549 cells used in binding studies were removed from the plastic by scraping with a rubber policeman, washed in Tris-Saline, and resuspended to 10^8 cells/ml in Tris-Saline.

[^{125}I]-IFN binding to cells was done according to the method of Stanley and Carver (9) with the modifications previously described (7). Briefly, [^{125}I]-IFN was preincubated at 0°C alone in 0.01 M Tris-HCl-0.15 M NaCl, pH 7.2 (Tris-Saline) or with lanthanide in the same buffer. 10^6 cells, washed 3 times with Tris-Saline, were then incubated with IFN or IFN-lanthanide mixtures (final volume 100 μl) for 10 min at 0°C. Samples were then added to microfuge tubes containing 280 μl of Tris-Saline with 15% BSA and centrifuged for 8 min. The tubes were rapidly frozen and the cell pellet separated from the supernatant by cutting off the bottom of the tube. Labeled IFN in the supernatant and bound to the cells in the pellet were counted separately and the percent bound [^{125}I] calculated from these numbers for each sample.

$LaCl_3 xH_2O$ (99.997%) and $YbCl_3 xH_2O$ (99.999%) were obtained from Apache Chemicals, Seward, IL.

RESULTS

Binding of the [^{125}I]-IFNs to cells was determined at 0°C to eliminate apparent binding that would result from internalization of IFN at physiological temperatures. The data presented in Table 1 indicate that enhanced binding of both human and mouse IFN-βs to homologous cells occurs at 1 mM concentration of $LaCl_3$. In Table 2 are shown the results of

TABLE 1. Effect of concentration of $LaCl_3$ upon binding of [^{125}I] human and mouse IFN-βs to homologous cells

	Mouse L cells		Human A549 cells	
[$LaCl_3$]	cpm cell bound[a)]	% bound	cpm cell bound[b)]	% bound
0	199[c)]	4.5	404	1.1
0.001 mM	136	2.9	247	0.6
0.01 mM	202	4.5	98	0.3
0.1 mM	197	3.5	402	1.0
1 mM	542	11.9	4,767	12.1

a) L cells incubated with 0.8 ng (1.62×10^{-3} μCi) of [^{125}I]-MuIFN-β.
b) A549 cells incubated with 1.25 ng (3.5×10^{-3} μCi) of [^{125}I]-HuIFN-β.
c) Each value is the mean of 3 independent determinations.

binding of [^{125}I]-HuIFN-β to human A549 cells in the presence of the lanthanides $LaCl_3$ and $YbCl_3$. In the absence of added lanthanides, 1.6 -

TABLE 2. Binding of lanthanide treated [^{125}I]-HuIFN-β to human A549 cells

Exp.	Addition	cpm cell bound[a)]	% bound[b)]	Fold increase in binding[c)]
1.	None	559	1.6	1
	100-fold excess unlabeled HuIFN-β	67	0.003	-
	1 mM $LaCl_3$	8,228	23.2	14
	2 mM $LaCl_3$	10,197	30.2	19
	1 mM $YbCl_3$	11,627	31.5	20
2.	None	1,219	3.3	1
	2 mM $YbCl_3$	17,404	49.4	17
	2 mM $YbCl_3$ + 100-fold excess unlabeled HuIFN-β[d)]	14,562	42.3	15
3.	None	1,376	2.1	1
	100-fold excess unlabeled HuIFN-β	35		-
	2 mM $LaCl_3$	24,026	36	17
	2 mM $LaCl_3$ + 100-fold excess unlabeled HuIFN-β[d)]	10,197	15.3	7
	2 mM $LaCl_3$ + 100-fold excess unlabeled HuIFN-β + 2 mM $LaCl_3$[e)]	46	0.04	-

a) A549 cells incubated with 1.25 ng (3.5×10^{-3} μCi) of [^{125}I]-HuIFN-β. All values a mean of 3 determinations.
b) The % bound calculated for all samples as the cpm cell bound divided by the total of the cell bound plus the supernatant for each sample.
c) Fold increase calculated using the % bound data.
d) The labeled IFN was mixed with 2 mM $YbCl_3$ or 2 mM $LaCl_3$ for 30 min and then added to cells along with unlabeled IFN not treated with lanthanide.
e) The labeled IFN was mixed with 2 mM $LaCl_3$ for 30 min and then added to cells along with 100-fold excess of unlabeled IFN that had been treated with 2 mM $LaCl_3$.

TABLE 3. Cross-species binding of [^{125}I]-HuIFN-β and [^{125}I]-HuIFN-β and cross-species antiviral activity of human and mouse interferons treated with $YbCl_3$

	cpm[a] (%) bound to cells		Antiviral titer[b] (IU/ml)	
Interferon	A549	L cells	A549	L cells
HuIFN-β	1,218 (3.3)	67 (0.18)	$2x10^6$	$2.4x10^3$
HuIFN-β + 2 mM $YbCl_3$	17,404 (47.2)	9,470 (25.66)	$5.8x10^6$	$1.7x10^3$
MuIFN-β	57 (1.1)	213 (4.1)	<200	$1.9x10^6$
MuIFN-β + 2mM $YbCl_3$	1,493 (29)	1,375 (26.6)	<200	$7x10^6$

a) 1.25 ng (3.5×10^{-3} μCi) of [^{125}I]-MuIFN-β or 0.8 ng (1.6×10^{-3} μCi) of [^{125}I]-HuIFN-β incubated with cells and binding determined. All values the mean of 3 determinations.

b) Unlabeled human or mouse IFN was incubated for 30 min at 0° with $YbCl_3$, and then the preparations assay for antiviral potency.

3.3% of the added [^{125}I]-IFN is bound to the A549 cells and this binding can be reduced to background levels by addition of 100-fold excess of unlabeled IFN. If the IFN is pretreated with 2 mM $LaCl_3$ or $YbCl_3$ for 30 min at 0°C prior to adding to cells, the quantity of [^{125}I] detectable in the pellet increases as much as 24 fold. Unlike untreated [^{125}I]-HuIFN-β whose binding is greatly reduced by unlabeled IFN, the lanthanide treated [^{125}I]-HuIFN-β binding was not reduced by unlabeled IFN. However, if unlabeled IFN is pretreated with $LaCl_3$, it then competes with $LaCl_3$ treated [^{125}I]-HuIFN-β for binding sites on the A549 cells.

There is very little cross-species binding of [^{125}I]-HuIFN-β and [^{125}I]-MuIFN-β (Table 3). In the presence of 2 mM $YbCl_3$ there is a substantial amount of heterologous IFN binding to the cells, however, this increased binding of heterologous IFN does not increase the level of cross-species antiviral activity.

DISCUSSION

We previously reported that lanthanide ions at concentrations of 2 mM or greater enhanced the antiviral activity of some human and mouse IFNs in

addition to protecting some IFNs, most notably HuIFN-β, from inactivation at 37°C (1). Since binding of IFNs to cell surface receptors is essential for biological activity (reviewed in 10), we used pure HuIFN-β and MuIFN-β labeled with [^{125}I] to assess whether the enhanced biological activity of IFNs treated with lanthanide salts results from increased binding of IFNs to cells. We examined binding at 0°C so that ligand-receptor binding could be distinguished from internalization of the IFN.

Both [^{125}I]-IFNs that we used in this study demonstrated specific binding to homologous cells since the amount of [^{125}I] bound to homologous cells was reduced by 100-fold excess of unlabeled IFN. $LaCl_3$ and $YbCl_3$ at concentrations of 1 mM or greater increase the binding of [^{125}I]-HuIFN-β and [^{125}I]-MuIFN-β to homologous cells by 20-30 fold. This is the range of concentration of lanthanides observed to enhance the antiviral activity of these IFNs (1).

Unlabeled native IFN does not compete with $LaCl_3$ treated [^{125}I] IFNs for receptor sites unless the unlabeled IFN has also been treated with $LaCl_3$. This can be interpreted to mean that $LaCl_3$ treated IFNs do not bind to the same receptors as do native IFNs. The fact that there is a 20-30 fold increase in binding but only a 2-5 fold increase in biological activity also suggest that most of the binding in the presence of lanthanides is not to specific high affinity IFN receptors. The lack of specific binding is also demonstrated by the greatly enhanced cross-species binding of both IFNs in the presence of the lanthanides. Although equivalent amounts of labeled IFNs are bound to homologous and heterologous species of cells, there is a stimulation of antiviral activity only in the homologous cell species. Generally, the lack of cross-species activity and also resistance to IFNs have been attributed to the lack of specific binding of IFNs to cells (10). However, there is significant binding of some IFNs to cells in which they exert no biological activity (11-13). The subsequent development of antiviral resistance in sensitive cells may result from the interaction of the bound IFN with a second membrane-associated component

(14,15) or may depend upon the proper intracellular processing of the IFN (2). Anderson and Vilcek (16) by constructing conjugates of IFN and ricin toxin B chain have demonstrated that HuIFN-α can exert an antiviral effect after binding to the receptor of another ligand (ricin toxin B chain). Thus it may be that the stimulation of antiviral activity of lanthanide treated IFNs in homologous cells results from interaction of bound IFN with a second membrane component after the enhanced initial nonspecific binding in the presence of the lanthanide or the lanthanides may stimulate intracellular processing of the IFN. If the necessary specific second component is not present in the heterologous cells or the cells do not process the IFN properly, even with high levels of binding of heterologous IFN, there is no stimulation of the sequence of events that make the cell resistant to viruses.

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