Acta virol. **37**: 509-514, 1993

COMPARISON OF ANTIGENIC PROPERTIES OF THREE INTERFERON (IFN)-ALPHA2 SUBVARIANTS AND ESTABLISHMENT OF A QUANTITATIVE IFN-ALPHA2 ELISA

G. KARAYIANNI-VASCONCELOS, N. FUCHSBERGER, Z. ŠKAMLOVÁ, P. KONTSEK

Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 842 46 Bratislava, Slovak Republic

Received February 8, 1993

Summary. - Three subvariants of human IFN-alpha2 (2a, 2b, 2c) were found antigenically highly homologous, using a panel of specific monoclonal antibodies (MoAbs) directed to seven epitopes. Only in the region 30-41 IFN-alpha2c showed some difference from the corresponding structures of subvariants 2a and 2b. An universal sandwich ELISA for the quantification of all subvariants of human IFN-alpha2 was designed. A major increase in sensitivity of the immunoassay could be achieved, when polyclonal antibody to IFN-alpha2 was combined with a mixture of three MoAbs to distinct sites of IFN-alpha2, compared to the combination of a polyclonal antibody with a single MoAb. The sensitivity of the established ELISA ranged between 1-10 units/ml of IFN-alpha2 and no cross-reactivity with IFN-alpha1, -beta, or -omega1 could be observed. We estimated the content of IFN-alpha2 to about 56 % in leukocyte IFN-alpha or to about 72 % in Namalwa IFN-alpha.

Key words: interferon-alpha2 subvariants; monoclonal antibodies; ELISA

Introduction

Virus-induced human IFN represents a mixture of at least 15 related subtypes with IFN-alpha1 and IFN-alpha2 as the main species (Hiscott *et al.*, 1984; Weissmann and Weber, 1986). Enzyme immunoassays (ELISA) for natural IFN-alpha cross-react with IFN-alpha1 and IFN-alpha2 and are therefore not suitable for subtype-specific determinations (Shearer *et al.*, 1984; Berthold *et al.*, 1985; Meager and Berg, 1986; Notani *et al.*, 1989). Moreover, three subvariants of human IFN-alpha2, designated 2a, 2b and 2c are known, which display also some differences in antigenic properties (Von Gabain *et al.*, 1990). In this paper we describe a specific sandwich ELISA for quantification of all alpha-2-subvariants, employing polyclonal antibody and MoAbs to IFN-alpha2 as a first

and second antibody, respectively. We also demonstrated an increased sensitivity of this assay resulting from application of a mixture of MoAbs instead of a single one.

Materials and Methods

Recombinant human interferons. IFN-alpha1, IFN-alpha2a (Roferon), IFN-alpha2c, IFN-omega1, hybrid IFN-omega1/alpha2 (66 N-terminal amino acids (aa) omega1 / 101 C-terminal aa alpha2c), hybrid IFN-alpha2/omega1 (64 N-terminal aa alpha2c / 108 C-terminal aa omega1) were kindly provided by Prof. G. Bodo and Dr. G. Adolf (Boehringer, Vienna, Austria). IFN-beta was kindly supplied by Bioferon (Laupheim, Switzerland). IFN-alpha2b and IFN-gamma were a gift from Prof. V. P. Zavyalov (Institute of Immunology, Lyubuchany, Russia). IFN-alpha2b with additional four amino acids (Asn-Glu-Phe-Met) at the N-terminus was prepared in this Institute (Kúdela *et al.*, 1992).

Antibodies to human IFN-alpha2. Polyclonal antibodies were produced by immunization of a sheep with recombinant IFN-alpha2c. The immunoglobulin (Ig) fraction of sheep antisera was isolated on DEAE-cellulose (Harlow and Lane, 1988). The neutralizing titer of purified Ig against 10 IU/ml IFN-alpha2 was 10^6 (data not shown).

Preparation and characterization of the mouse MoAbs to IFN-alpha2 was described previously (Novák *et al.*, 1986; Kontsek *et al.*, 1991). These MoAbs were used as culture supernatants or purified Ig from ascitic fluid by protein A-affinity chromatography (Harlow and Lane, 1988).

IFN-bioassay. The antiviral activity of IFN was determined by the cytopathic effect inhibition method on bovine MDBK cells challenged with vesicular stomatitis virus (VSV) in microtiter plates. Medium RPMI 1640 supplemented with 5 % foetal calf serum was used for cell cultivation and IFN and VSV dilution. All samples were investigated in triplicate in two independent determinations. IFN titers were expressed in international units (IU) per ml, using IFN-alpha Standard G 032-901-527 supplied by the National Institutes of Health (Bethesda, USA).

IFN-alpha2-sandwich ELISA. Immunoplates (Koh-i-noor, Czecho-Slovakia) were coated at 37 °C overnight with 50 μl of sheep anti-IFN-alpha2 Ig diluted 1:4000 in phosphate-buffered saline (PBS, pH 7.2) per well and then blocked with 1 % nonfat dry milk.

(a) Determination of MoAb-reactivity with IFN-alpha2-subvariants: Fifty μl of IFN (10^4IU/ml) were added into coated wells for 1 hr at 37 °C. Plates were washed and incubated with 50 μl/well of the MoAb-containing culture supernatants for another 1 hr at 37 °C. Bound molecules were detected with peroxidase-conjugated swine anti-mouse Ig (ÚSOL, Prague, Czecho-Slovakia). After washing the reaction was visualized with orthophenylenediamine (ODP) and absorbance at 492 nm was read using Multiscan MCC/340 (Labsystems, Finland).

(b) Quantitative immunoassay: Different concentrations of IFN diluted in PBS were added into coated wells in 50 μl amounts for 1 hr at 37 °C. After washing 50 μl/well of either single purified MoAb (1 μg/ml) or mixture of three MoAbs (1:1:1, v:v) were added as a second antibody in 50 μl/well. Following incubation for 1 hr at 37 °C, the bound MoAbs were detected with labelled anti-mouse Ig, the reaction developed with OPD and the A_{492} measured. The value of nonspecific binding of antibodies (in wells containing dilution buffer instead of IFN) was substracted from readings. Each sample was assayed in quadruplicates in three independent determinations.

Results

Binding of MoAbs to IFN-alpha2 subvariants

A panel of MoAbs raised to human IFN-alpha2b or to human IFN-alpha2c was characterized according to the reactivity with the subvariants -alpha2a,

Table 1. Reactivity of MoAbs with IFN-alpha2 subvariants in ELISA

MoAb	Epitope	IFN					
		alpha				alpha2c/ omega1	omega1/ alpha2c
		2a	2b	2c	2b+5aa		
4-1	1-64	++	++	++	++	+	-
7-1	1-64	+++	+++	+++	+++	++	-
N40	30-41	++	++	++	++	+	-
N43	30-41	+	+	++	+	+	-
N7	36-38	+	+	+++	+	-	-
N27	43-54	+++	+++	+++	+++	++	++
N12	63-76	++	++	++	++	ND	++
N8	77-85	++	++	++	++	ND	+
N54	105-125	+++	+++	+++	+++	-	++

A_{492} values: + (>0.2), ++ (>0.4), +++ (>0.6)
ND: not determined

-alpha2b and -alpha2c in a sandwich ELISA (Table 1). Moreover, two preparations of IFN-alpha2b were tested - a full-size protein and that with the additional five amino acids at the N-terminus. Antibodies directed to six different epitopes of IFN-alpha2 exerted approximately the same binding with four recombinant IFN-preparations. Only MoAbs N7 and N43 with the binding sites located around residues 30–41 showed the highest reactivity towards subvariant 2c (the antigen used to establish these MoAbs).

Sandwich ELISA for quantification of IFN-alpha2

For the quantitative IFN-alpha2-ELISA the MoAbs 7-1, N27 and N54 with the strong binding to all alpha2-subvariants and recognizing different sites were selected. To determine the optimal sandwich configuration of the assay, the first polyclonal Ig to IFN-alpha2 was combined with purified MoAbs at the concentration 1 μg/ml. Combinations using single MoAbs did not demonstrate significant differences between three compared antibodies and the sensitivity of these immunoassays for IFN-alpha2 averaged at 50–100 IU/ml. However, when MoAbs 7-1, N27 and N54 were mixed at a ratio 1:1:1 and this cocktail was used as a second antibody, an about 5-fold increase in the sensitivity of the assay could be observed (Fig. 1). At this configuration, the ELISA was able to detect less than 10 IU/ml of IFN-alpha2. Application of MoAbs in the concentration range 0.1–10 μg/ml did not significantly affect the sensitivity of the immunoassay (data not shown).

Assay of natural IFN-alpha2

The developed ELISA was used for estimation of IFN-alpha2 content in

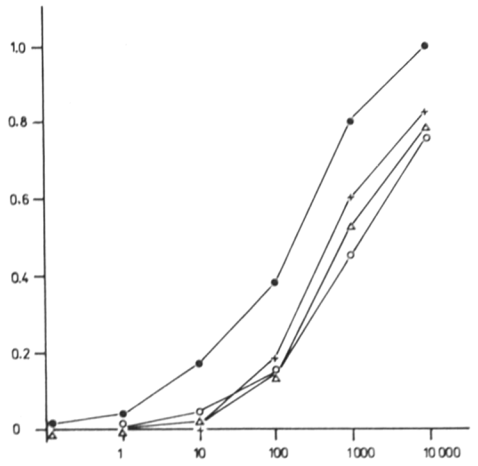

Fig. 1
Standard curves of human IFN-alpha2 in sandwich ELISA
As a second antibody either single MoAbs or cocktail of three MoAbs were used. Means of quadruplicate determinations are shown. Standard deviation was less than 10 % of the mean.
Abscissa: IFN-alpha2 (IU/ml); ordinate: A_{492}. (-○-) MoAb 7-1; (-x-) MoAb N27; (-△-) MoAb N54; (-●-) MoAb-mixture.

unpurified preparations obtained from Newcastle disease virus (NDV)-induced human leukocytes or Namalwa cells. Two samples of leukcoyte IFN-alpha and two samples of Namalwa IFN-alpha were analyzed. The antiviral titers of preparations were compared with the corresponding ELISA-titers of IFN-alpha2 (Table 2). We found that IFN-alpha2 could contribute by about 54–56 % and 68–72 % to the total antiviral activity of leukocyte or Namalwa IFN-alpha, respectively.

Table 2. Relative frequency of IFN-alpha2 in natural human IFN-alpha

Sample	NDV-induced IFN-alpha	Bioassay IU/ml	IFN-alpha2 ELISA	
			IU/ml (mean ± SD)	% of total activity
1	leukocyte	512	295 ± 25	58
2	leukocyte	1024	550 ± 48	54
3	Namalwa	256	175 ± 15	68
4	Namalwa	128	97 ± 8	76

SD: standard deviation

Discussion

Human IFN-alpha2 is one of the two major species which are present in preparations of natural IFN-alpha. There are three IFN-alpha2 subvariants - 2a, 2b and 2c, which differ by only one or two amino acids from each other at positions 23 and/or 34 and these minor changes determine also antigenic differences between these proteins. Using MoAbs directed to seven different sites of IFN-alpha2, we found the antigenic structures of alpha2-subvariants to be highly homologous. Only in the region 30–41 we observed some differences of IFN-alpha2c (arginine at position 34) from IFN-alpha2a and IFN-alpha2b (histidine at position 34). The additional five amino acids at the N-terminus did not influence the antigenic properties of IFN-alpha2 protein.

Usually an antibody pair is involved in sandwich assays - a first antibody (polyclonal or monoclonal) used for coating is combined with a second antibody for binding of captured antigen. When polyclonal antibody to IFN-alpha2 was combined with the single MoAb to IFN-alpha2, the sensitivity of these assays averaged at 50–100 IU/ml. In our recent work we found that a mixture of MoAbs to IFN potentiated the efficacy of the neutralizing bioassay. Based on this observations we expected, that also in the immunoassay the MoAbs recognizing distinct antigenic sites could bind simultaneously to one IFN-alpha2 molecule and thus to intensify the detection signal. Actually, when polyclonal Ig for coating was combined with cocktail of three MoAbs about 5-fold increase in the sensitivity of the assay (1–10 IU/ml) could be achieved. The cross-reactivity with IFN-alpha1, -beta, -gamma and -omega1 was not observed.

The established ELISA, in contrast to bioassay, permitted a rapid assessment of IFN-alpha2 in preparations of natural IFN-alpha. Because of low frequency of other (not tested) alpha-subtypes in such preparations, the theoretical cross-reactivity with some of them should not substantially affect the specificity of IFN-alpha2 determination. Therefore we supposed that subtype alpha2 might be responsible for approximately 56 % of the antiviral activity of leukocyte IFN-alpha and for about 72 % of activity of lymphoblastoid Namalwa IFN-alpha.

Acknowledgement. This work was supported by the Slovak Academy of Sciences, Grant GA-SAV 225/1991.

References

Berthold, W., Merk, W., and Adolf, G. R. (1985): Immunoassay for the quantitation of human leukocyte interferon. *Drug Res.* **35**, 364–369.

Harlow, E., and Lane, D. (1988): *Antibodies. A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Hiscott, J., Cantell, K., and Weissmann, C. (1984): Differential expression of human interferon genes. *Nucleic Acids Res.* **12**, 3727–3746.

Kontsek, P., Borecký, L., Kontseková, E., Máčiková, I., Kolcunová, A., Novák, M., and Krchňák, V. (1991): Mapping of two immunodominant structures on human inteferon alpha2c and their role in binding to cells. *Mol. Immunol.* **28**, 1289–1297.

Kúdela, O., Zelník, V., Kaluz, Š., and Pastorek, J. (1992): The use of consensus sequence for

amplification and oriented cloning of human alpha interferon genes. *Acta virol.* **36**, 488–490.
Meager, A., and Berg, K. (1986): Epitope localization of a monoclonal antibody, LO-22, with broad specificity for interferon-alpha subtypes. *J. Interferon Res.* **6**, 729–736.
Notani, J., Kaizu, T., Mutoh, S., Otsuka, K., Kusunoki, Ch., Yamada, H., Nagayoshi, A., Suzuki, S., Niwa, M., and Ueda, I. (1989): Application of Namalwa inteferon-alpha monoclonal antibodies for purification and enzyme immunoassay of inteferon-alpha. *J. Immunoassay* **10**, 257–276.
Novák, M., Borecký, L., Kontseková, E., Kontsek, P., and Nováková, M. (1986): Preparation and characterization of hybridomas producing monoclonal antibodies against human alpha interferon. *Acta virol.* **30**, 228–233.
Shearer, M., Taylor-Papadimitriou, J., Griffin, D., and Balkwill, F. (1984): Monoclonal antibodies that distinguish between subspecies of human interferon-alpha and that detect interferon oligomers. *J. Immunol.* **133**, 3096–3101.
Von Gabain, A., Lundgren, E., Ohlsson, M., Holmgren, E., Josephsson, S., and Alkan, S. S. (1990): Three human interferon-α2 subvariants disclose structural and functional differences. *Eur. J. Biochem.* **190**, 257–261.
Weissmann, C., and Weber, H. (1986): The interferon genes. *Progr. Nucleic Acids Res. mol. Biol.* **33**, 251–300.