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Characteristics of Autoantibodies to Human Interferon in a Patient with Varicella-Zoster Disease

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A patient with varicella-zoster disease was found to have antibodies to human interferon. These antibodies included all IgG subclasses, showed a high combined affinity (average dissociation constant, $10^{-11}M$), and were present in serum at a concentration of $10^{-9}M$. The antibodies neutralized the activity of a series of human α -interferons prepared from recombinant DNA as well as that of naturally occurring mixtures of α -type interferon prepared by viral stimulation of human cells. Examination of the patient's lymphoid cells revealed normal production of interferon and normal expression of interferon receptors. Interferon from the patient's cells was neutralized by her serum. The neutralizing capacity of this serum was analyzed with regard to levels of interferon previously detected in patients with varicella-zoster infections.

To date, nine patients with antibody to human interferon [1] have been described [2-6]. In four instances the antibodies appeared before or without clinical administration of interferon. In this study we examined the nature of these antibodies in one patient with respect to their specificity and capacity to block the activity of circulating interferon. We also attempted to estimate what kind of threat such antibodies might pose to a functional interferon system.

The patient studied (designated patient VZ) [3]

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had recovered from a generalized eruption caused by varicella-zoster virus and had remained free of acute viral infections for one year while maintaining the same levels of circulating antibodies to interferon that had been present during (and possibly before) the transformation from localized to generalized disease. The day after dissemination became obvious, she received a single im injection of leukocyte interferon. No plasma interferon was subsequently detected; as shown in this report, the neutralizing and binding capacities of this patient's antibody to interferon (which was already present in a sample taken before the injection of interferon) were quite sufficient to inhibit plasma interferon activity after a dose of the size administered.

Materials and Methods

Patient. On October 6, 1980, a healthy 77-year-old woman with no history of frequent viral infections acquired typical herpes zoster localized to the fourth-lumbar-nerve distribution; five days later a generalized varicella eruption developed. On the sixth day a single injection of human leukocyte interferon was given to the patient. A blood sample obtained before injection was later found to contain antibody to interferon. Apart from the episode of varicella-zoster disease, which cleared within three weeks [3], there was no indication of overt or underlying disease in this patient.

Antibody. Antibody to interferon was assayed in plasma or serum samples from patient VZ. Neither the presence of heparin as an anticoagulant in

the plasma samples nor heat decomplexation affected the results of any assay. The first sample obtained from the patient was a serum sample; this specimen was taken on October 12, 1980, prior to the single injection of interferon. The last sample obtained was a plasma sample; this specimen was collected on November 12, 1981. Control preparations of human serum and plasma were obtained from a healthy volunteer. For purposes of comparison, rabbit antiserum to human interferon BK3 [7] was also included in several assays.

Cells. The final plasma sample was separated from cellular components, which were fractionated on a discontinuous Ficoll gradient (Pharmacia HB, Uppsala, Sweden) [8]. Lymphocytes were washed and resuspended in RPMI-1640 (GIBCO-Europe, Paisley, Scotland) supplemented with 10% fetal calf serum. A portion of this suspension was used for receptor binding studies, a portion for interferon induction by Newcastle disease virus (NDV), and a portion for transformation by Epstein-Barr virus (EBV).

Transformation by EBV. Peripheral-blood lymphocytes, isolated on a discontinuous Ficoll gradient, were exposed for 1 hr to EBV (strain B95-8, purified by sucrose density centrifugation) at a multiplicity of infection of 1.0, according to the method of Henle et al. [9]. After several serial passages, a rapidly multiplying cell line positive for EBV nuclear antigen was established.

Interferon induction by NDV. Both peripheral-blood lymphocytes and established lines of EBV-transformed B cells were induced with NDV to produce interferon. Cells were resuspended at a concentration of 10^6 /ml and were treated with NDV (at a final HA titer of 800) for 18 hr at 37°C, according to previously described techniques [10].

Interferon preparations. The preparations of interferon used are described in table 1. The nomenclature and abbreviations used follow the guidelines for the main serotypes, α , β , and γ [1]. In the table we have listed the common abbreviations for species produced from recombinant DNA [13]. Herein we use the subscript system of our supplier, Prof. C. Weissmann (University of Zurich) [13]. Except for IFN α_1 and IFN α_2 , all of the "recombinant" interferon preparations were in the form of crude extracts from *Escherichia coli*. The IFN α_1 preparation was ~10% pure, and the IFN α_2 preparation was >50% pure [12]. A mixed interferon preparation from virus-stimulated Namalwa cells (IFN α -ly) was purified as has been described

previously [14]. This material was a gift from Dr. K. Fantes (Burroughs Wellcome, Beckenham, Kent, UK) and had been used in the original identification of antibodies to interferon in patient VZ [3]. IFN β was prepared from human foreskin fibroblasts as described previously [15] and was used in unpurified form. Interferon prepared from the leukocytes of healthy donors (IFN α -leuk) was obtained from Dr. K. Cantell [16].

Titration of interferon. Antiviral titers were determined by tests for 50% protection against the CPE of vesicular stomatitis virus (multiplicity of infection, 0.001) in either human neuroblastoma cells (T98G) [17] or Madin-Darby bovine kidney (MDBK) fibroblasts [18]. The potency of interferon was expressed relative to the international reference MRC 69/19 [19]. For all antiviral titers given here, the degree of precision at the 95% level was $\pm 0.5 \log_{10}$ [17].

Neutralization of interferon. Neutralization experiments were performed by the titration of serial dilutions of antibody against serial dilutions of interferon in an antiviral assay. Serum and interferon were incubated together for 30 min at room temperature before being added to cells. End points were scored at 50% CPE. The interferon types to be neutralized were titrated separately in the same assay. Neutralizing titers were calculated as follows: 1/antibody dilution at 50% CPE \times interferon titer neutralized. The degree of precision was the same as that for the interferon titration assay.

RIA. An RIA was performed as previously described, with *Staphylococcus aureus* (strain Cowan I) as a solid phase [17]. Optimal dilutions of antiserum (final dilution, 1:1,000) were chosen for the display of maximal antibody affinity.

Receptor binding. The procedure used for receptor binding was exactly as that described for peripheral-blood lymphocytes [8]. A labeled IFN α -ly preparation from Namalwa cells was used since our earlier data on normal lymphocyte binding had been obtained with this type of interferon.

Radiolabeled preparations of interferon. Interferon preparations were labeled with ^{125}I as has been described previously [14, 20, 21]. Molar concentrations of interferon have been used in other investigations for the calculation of dissociation constants for cellular receptors or for antibody-interferon complexes; these values were calculated in this study from the specific molar incorporation of ^{125}I into biologically active interferon. The de-

Table 1. Designation, composition, and titer of human interferon (IFN) preparations.

Designation	Alternative nomenclature	Composition	Relative antiviral titer (units/ml)* on indicated cell type	
			Human neuroblastoma cells	Bovine kidney fibroblasts
IFN α -leuk	Leukocyte IFN, C-IF, P-IF [11]	Mixed α species, minor β component [12]	10,500	10,500
IFN α -ly	Lymphoblastoid IFN [11] (Namalwa IFN) [†]	Mixed α species, 10%–20% β components [12]	10 ⁷	3 × 10 ⁷
IFN α_1	IFN LrD	Homogeneous gene products from recombinant DNA [13]	9 × 10 ⁵	6 × 10 ⁷
IFN α_2	IFN LrA		2 × 10 ⁷	10 ⁷
IFN α_4	IFN LrE		6 × 10 ⁵	10 ⁶
IFN α_6	?		3 × 10 ⁶	8 × 10 ⁵
IFN α_7	?		8 × 10 ⁴	2 × 10 ⁵
IFN α_8	IFN LrB		3 × 10 ⁵	10 ⁵
IFN α -leuk VZ [‡]	120	60
IFN α -ly VZ [‡]	800	240

* Titers indicate relative potency against a laboratory reference preparation that is fixed at 10,500 units/ml with respect to the international reference MRC 69/19. Except for those obtained from patient VZ, all preparations titrated were in purified or concentrated crude form.

[†] With few exceptions, the sole commercial source of this preparation is the Namalwa Burkitt line [11].

[‡] This preparation, obtained from patient VZ, was crude and unconcentrated.

tails of these calculations are presented elsewhere for IFN α -ly (Namalwa IFN) [14] and for IFN α_2 [20]. Typically, 75%–80% of the radioactivity of the preparation was present in interferon protein, 5%–10% was present as acid soluble ¹²⁵I, and 15% was present as a component that migrated separately from interferon on electrophoresis but did not appear to be associated with any major contaminant.

In experiments where ¹²⁵I-labeled α -interferon was bound to either cell receptors or antibodies, specific binding was calculated by subtraction of the residual radioactivity, in the presence of an excess concentration of unlabeled interferon, from total bound radioactivity. These background values are always linearly proportional to the "input" radioactivity. In cell binding experiments, the values are ~0.10% of the input radioactivity; in RIA experiments, 0.3%; and in immunoprecipitation experiments, 0.4%. The displacement of radioactivity by unlabeled preparations of interferon is always directly proportional to the amount of unlabeled interferon used and independent of the purity of the preparations.

Labeled interferon has been characterized previously [14, 17, 20–23]. According to all tests available to us, ¹²⁵I-labeled α -interferons behave as do their unlabeled forms. The 95% confidence limits for the estimation of interferon molarity (from a

mean of 100% radioactive incorporation) are 80%–125% [20].

IgG subclass specificity. Subclass-specific sheep antisera to human myeloma IgG subclasses 1, 2, 3, and 4 were obtained from the World Health Organization IUIS; Immunoglobulin Subcommittee, Bern, Switzerland [24]. The relative potency of these antisera was titrated by direct RIA against a sample of ¹²⁵I-labeled human IgG prepared from the serum of a healthy donor. Immune coprecipitation of ¹²⁵I-labeled IFN α_2 was performed with an additional rabbit antibody to sheep IgG. Non-specific precipitation was estimated by inclusion of a 2,000-fold excess concentration of unlabeled IFN α_2 in control experiments. Sera were added to 50 μ l of ¹²⁵I-labeled IFN α_2 (250 units/ml) in the following order and proportion: human serum (from patient VZ), 1 μ l; sheep serum, 10 μ l; and rabbit serum, 225 μ l. The final volume was 500 μ l. After incubation for 15 days at 4 C, precipitates were washed twice with 1 ml of buffer and counted for radioactivity.

Results

The results of interferon titration experiments are shown in table 1. The titer of IFN α_1 in human cells was 1.5% of that measured in MDBK cells. Also shown are the interferon titers in cells obtained

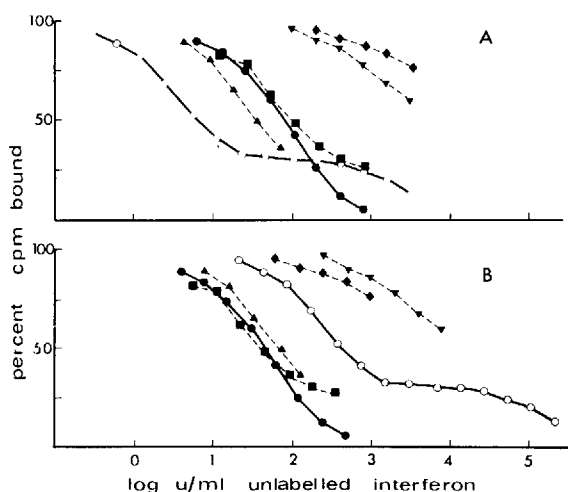


Figure 1. Curves for competitive binding by unlabeled human α -interferons and ^{125}I -labeled IFN α_2 (25 units/ml) to antibody to interferon from patient VZ, with *S. aureus* as a solid phase. Labeled and unlabeled interferons were premixed in a buffer containing 0.2% bovine serum albumin. Plasma from patient VZ was added to a final dilution of 1:900, and the mixture (450 μl) was incubated overnight at room temperature. *S. aureus* (50 μl) was added to a final concentration of 0.05% (vol/vol). After incubation for 1 hr at room temperature, *S. aureus* was pelleted, washed twice, and counted for bound radioactivity. The "input" radioactivity was $10,155 \pm 46$ cpm (12.5 units, 3.1 fmol); the total bound radioactivity in the absence of unlabeled interferon (100%) was $1,256 \pm 13$ cpm; and the background radioactivity (0) was 32 ± 1 cpm. The final titer of unlabeled interferon, as determined in human cells (A) and in bovine cells (B), is represented by the following symbols: IFN α_1 , \circ ; IFN α_2 , \bullet ; IFN α_4 , \blacktriangle ; IFN α_6 , \blacksquare ; IFN α_7 , \blacktriangledown ; and IFN α_8 , \blacklozenge .

from patient VZ after NDV stimulation. These values were within the normal range for such cells [11, 16].

Figure 1 shows radioimmune competition curves for various species of α -interferon derived from recombinant DNA, with their concentrations based on titers obtained in human or MDBK cells. All species tested competitively inhibited the binding of ^{125}I -labeled IFN α_2 to antibody from patient VZ; only unlabeled IFN α_2 showed homogeneous displacement. The titer obtained in human cells clearly represented a gross underestimate of the real molar concentration of IFN α_1 . With the titer obtained in MDBK cells, the competition curve for IFN α_1 was displaced back to the right of that for IFN α_2 . With respect to their competitive capacities against ^{125}I -labeled IFN α_2 , the other species may be grouped in descending order of effectiveness, as

follows: (1) IFN α_4 and IFN α_6 , (2) IFN α_7 and IFN α_8 . A similar grouping for plasma neutralization titers was seen; against IFN α_2 , IFN α_4 , and IFN α_6 , the titer was $\sim 4,000$; against IFN α_7 and IFN α_8 , it was $\sim 1,000$; and against IFN α_1 , it was 5,000 in MDBK cells and 200 in human cells. The two interferon species from the patient's own cells (table 1) were neutralized by her plasma at a titer of $\sim 1,000$, as assayed in both human and bovine cells.

Figure 2 shows that lymphocytes derived from patient VZ possessed functional surface receptors for α -interferon that did not differ significantly from those normally seen on human lymphocytes. The confidence limits are those calculated from results previously obtained with a group of eight healthy volunteers [8]. The dissociation constant for ^{125}I -labeled IFN α -ly was 6×10^{-11} M.

Finally, some aspects of the production of antibodies to interferon by patient VZ were analyzed.

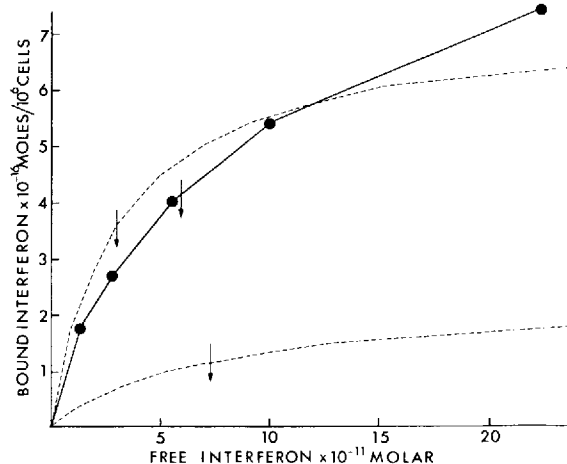


Figure 2. Curve for direct binding by ^{125}I -labeled IFN α -ly to peripheral-blood lymphocytes from patient VZ. The dashed lines represent 95% confidence limits determined in a previous study [8] in which the binding of interferon to peripheral-blood lymphocytes from eight healthy donors was measured under similar conditions. The arrows intersecting each curve indicate the concentration of interferon that saturates half the sites, as determined by Scatchard analysis. The values shown for bound interferon represent receptor-specific binding, with non-specific background levels subtracted. In this assay 10^6 cells/ml were incubated for 1 hr at 37 C. The cells were then placed on ice and washed twice with cold medium, and the cell pellet was counted for radioactivity. For the highest concentration shown, the input radioactivity was 783,200 cpm; the total cell-bound radioactivity was $1,958 \pm 46$ cpm; and the background radioactivity was 50 ± 6 cpm.

Figure 3 shows curves for binding of ^{125}I -labeled $\text{IFN}\alpha_2$ to antibody from patient VZ in the presence of *S. aureus*. The two samples from which the antibody was derived were obtained more than a year apart. The similarity of the Scatchard plots shows that antibody production remained constant with respect to both quality and quantity. The proportional activities of $\text{IFN}\alpha_1$ and $\text{IFN}\alpha_2$ in the competitive binding assay were also the same in these two samples (data not shown). The inset shows antibody binding data in the form of a Scatchard plot. From the known incorporation of radioactivity, we used the slope and the abscissa intercept to calculate an average dissociation constant for $\text{IFN}\alpha_2$ at 10^{-11} M and concentration of IgG antibody to interferon at 10^{-9} M (a value that for normal human IgG would represent ~ 100 ng/ml).

Table 2 shows that the antibody to interferon de-

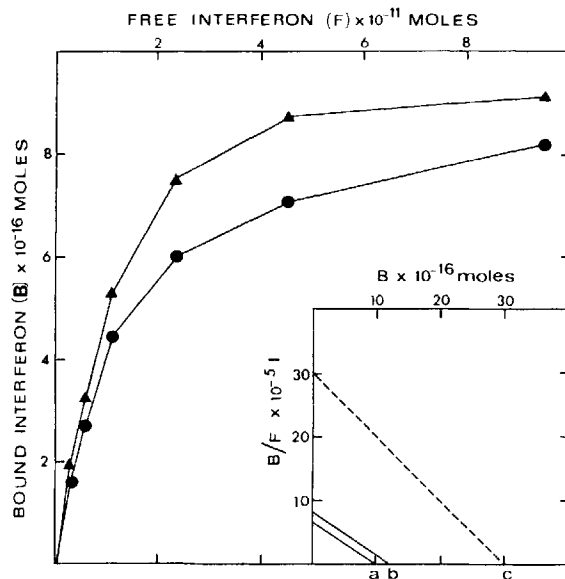


Figure 3. Direct binding of ^{125}I -labeled $\text{IFN}\alpha_2$ to *S. aureus* in the presence of two samples of antibody to interferon from patient VZ: that derived from plasma collected on November 12, 1981 (labeled \blacktriangle in the larger graph and "b" in the inset), and that derived from serum collected on October 12, 1980 (labeled \bullet in the larger graph and "a" in the inset). The inset shows Scatchard plots; the dotted line (c) represents the plot for a hyper-immune rabbit antiserum to human interferon (neutralizing titer, 1:20,000). The methods used were the same as those described for figure 1, with varied concentrations of ^{125}I -labeled $\text{IFN}\alpha_2$. For the highest concentration shown (380 units/ml, 9.5×10^{-11} M), the "input" radioactivity was 210,955 cpm; the total bound radioactivity was 3,536 and 3,876 cpm; and the background radioactivity was 633 ± 30 cpm.

Table 2. Reaction of interferon antibodies from patient VZ with sheep antisera to human IgG subclasses, as measured by immune-specific coprecipitation of ^{125}I -labeled $\text{IFN}\alpha_2$.

IgG subclass	Immunoprecipitation due to plasma of patient VZ		Relative RIA titer of IgG antibody to normal human IgG [†]
	Specific cpm in precipitate*	Relative titer [†]	
1	807 (<0.001)	14.1	4.7
2	57 (<0.01)	1.0	1.0
3	594 (<0.001)	10.4	13.8
4	65 (<0.05)	1.1	1.7

* For precipitated radioactivity, specific cpm values are equal to total precipitated cpm minus background cpm. Background values were obtained from radioactivity precipitated in the presence of a 2,000-fold excess of unlabeled $\text{IFN}\alpha_2$. *P* values (shown in parentheses) were determined by Student's *t* test for the difference between mean values in duplicate tests and are estimates of the significance level at which total cpm differed from background cpm. The "input" radioactivity was $15,327 \pm 53$ cpm; the background radioactivity was 60 ± 4 cpm.

[†] Relative titers are individual values/values for IgG₂.

tected in patient VZ included all four subclasses of IgG. Relative titers for specific RIA coprecipitation of ^{125}I -labeled $\text{IFN}\alpha_2$ are presented together with an independent estimation of the relative titers of the subclass-specific antisera. The ratios are comparable and show that the antibodies to interferon were distributed among the subclasses in more or less the ratios that are commonly found for the subclasses themselves.

Discussion

Our results may be summarized as follows: Patient VZ continuously produced polyclonal neutralizing antibody to α -interferons. Cells from the patient were capable of producing interferon in response to viral stimulation, and the antibody neutralized this interferon. The patient's own lymphocytes possessed normal functional receptors for α -interferon.

The polyclonal nature of the antibodies to interferon produced by patient VZ was inferred from (1) the biphasic cross-reactions of α -interferons competing with ^{125}I -labeled $\text{IFN}\alpha_2$ (figure 1); (2) the presence of antibody of all four IgG subclasses (table 2); and (3) the relatively broad specificity of neutralization combined with high avidity. The specificity of the antibodies extended to all species of human α -interferon tested, and the neutralizing

activity of the antibodies was effective against natural mixtures of interferon that were predominantly α in composition. Recent work has shown that IFN α , has a lower binding affinity for the interferon receptors of human cells than do the other α species and consequently has a lower level of specific activity in human cells [25, 26].

Cross-reaction with β -interferon was not detected by either competition or neutralization assays [3]. However, the apparent exclusion of β -interferon may be related to the limits of the assay; e.g., a partial reaction of undiluted serum with low concentrations of β -interferon is beyond the powers of resolution of the assay. This point is of interest because all naturally occurring mixtures of α -interferon contain detectable amounts of β -interferon [12], and immunologic cross-reaction usually occurs during the immunization of animals with leukocyte interferon [15]. We did not consider the role of γ -interferon in this study because, although it is a lymphokine that shares some of the biologic activities of α - and β -interferon, it makes use of a different cellular receptor [27].

In this study we posed the following question: How can interferon function in vivo in the presence of circulating antibodies to interferon? In the case of patient VZ, we cannot answer the question of whether or not interferon actually did function. It is possible that the patient recovered from her zoster infection and maintained a resistance to acute viral infections by means of defense mechanisms unrelated to interferon. Nevertheless, given that her ability to produce and to respond to interferon was not manifestly abnormal (table 1, figure 2), the question of how she coped is still relevant.

The question centers on the role of plasma interferon in viral infections. In many of the cases studied by various researchers, human antibody to human interferon has first been detected after a failure to find interferon in plasma or serum following an injection of interferon [3–5]. An examination of the values reported for circulating interferon either during viral disease [28, 29] or after im or sc injection [30] reveals that interferon titers in serum or plasma have rarely been found to exceed 1,000 units/ml. The data collected by Scott [30] show that the plasma pool for injected interferon has a tendency to become saturated as the dose increases. Similar results have been obtained in experiments with animals [31]. Constant levels of plasma interferon may occur only when the mean rates of entry from the extravascular pool match

the rates of catabolism of interferon; the plasma concentration would be limited by these rates [32].

It has long been recognized that interferon production in vivo is usually associated with the site and magnitude of viral multiplication (for a review, see [33]). Some clue as to the magnitude of local production can be found in conditions where physical barriers to the free diffusion of interferon exist. Overall et al. [34] found a mean of 20,000 units of interferon (predominantly the α form)/ml of vesicle fluid from lesions of herpes labialis. Armstrong et al. [35] found \sim 1,000–10,000 units of interferon/ml of vesicle fluid, with titers of circulating interferon of $<$ 10 units/ml, in cases of both herpes zoster and chickenpox. The latter authors found a positive correlation between interferon titers in vesicle fluid and the subsequent course of infection.

The titers just mentioned exceed by up to an order of magnitude the neutralizing capacity of plasma from patient VZ against a mixture of α -interferons. Gresser et al. injected sheep antibodies to murine interferon into mice and thereby exacerbated the course of several experimental viral infections [36, 37]. Insofar as it is possible to correlate the amount and avidity of the antibody injected with the amount and avidity of the antibody present in the plasma of patient VZ, the minimal doses needed to affect the course of infection in mice had a serum neutralizing capacity well in excess of that shown by the plasma of patient VZ.

It seems to us a reasonable conclusion that the amount of circulating antibody present in the plasma of patient VZ may have been too small to exert a great effect on the interferon response to viral infections outside the circulation. However, this conclusion must remain tentative since we do not know how much interferon was produced locally during the patient's zoster infection. The coincidence of neutralizing antibody with the dissemination of disease may indicate that in this instance the production of interferon was inadequate.

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