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Mouse Interferons: Amino Terminal Amino Acid Sequences of Various Species

Abstract. *Mouse interferons of three size classes (A, 35,000 to 40,000 daltons; B, 26,000 to 33,000 daltons; and C, 20,000 daltons) were purified from Ehrlich ascites tumor cells infected with Newcastle disease virus. The sequences of the first 24 amino acids (No. 17 has not been identified) of interferons A and B are identical. The sequence of the first 20 amino acids of interferon C differs from that of A and B in 18 positions. There is partial homology in amino terminal sequence between mouse interferons A (or B) and a human fibroblast interferon and between mouse interferon C and a human lymphoblastoid interferon.*

Interferons are glycoproteins that are produced by various vertebrate cells in response to viral infection or some other stimuli. They are released from the producing cells, interact with other cells, and alter the biochemical and immunological characteristics of these in various ways. Interferons are species specific: they are more active usually in homologous than in heterologous cells (*1*).

There are several reports on the purification of mouse interferons induced by infection with Newcastle disease virus in cultured mouse cells. The purified interferons cover a wide molecular weight range. Those isolated from L929 and C243 cells were found to consist of two species, a larger one of 35,000 to 40,000 daltons and a smaller one of 22,000 to 28,000 daltons (*2*). We reported earlier the isolation of mouse interferons from cultures of Ehrlich ascites tumor cells infected with Newcastle disease virus (*3*, *4*). These consist of three size classes: A, about 35,000 daltons; B, about 26,000 daltons, and C, about 20,000 daltons. The specific activity of each is about 2×10^9 NIH units per milligram of protein. A and B migrate as broad bands on polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS), and C gives a sharper band. The amino acid compositions of interferons A, B, and C are similar. The tryptic peptide maps of A and B are very similar, that of C is different (*3*, *4*).

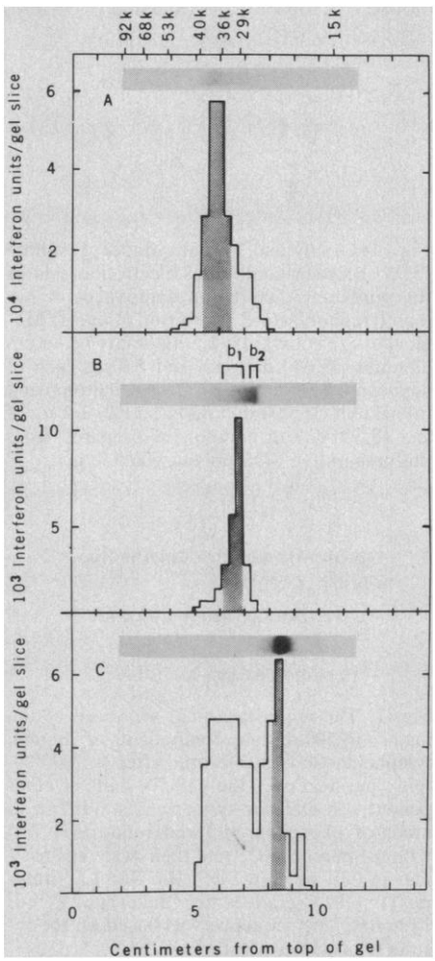

Fig. 1. Analyses by polyacrylamide gel electrophoresis in the presence of SDS of three protein fractions enriched in interferons, showing the size distributions of interferon active materials and of proteins. Panels A, B, and C show the analyses of preparations enriched in interferon classes A, B, and C, respectively. The horizontal inserts at the tops of the panels are photographs of the appropriate gel tracks after they were stained for proteins with Coomassie brilliant blue. For detection of interferon activity, the gel tracks were sliced, the slides were eluted, and the eluted material was tested in an assay based on the protection of cells treated with interferon from the cytopathic effect of vesicular stomatitis virus. The amino terminal amino acid sequences of the proteins eluted from the shaded regions of the gel tracks shown in panels A, B and C, and in the case of the gel track shown in panel B also, from the region b_2 were determined.

Here we report the NH_2-terminal amino acid sequences of mouse interferons from size classes A, B, and C. The detailed description of the improved and highly sensitive amino acid sequencing technique used to establish these has been provided by Hunkapiller and Hood (*5*).

The interferons were produced in Ehrlich ascites tumor cells and purified according to a modified version of our described procedure (*4*). This involves chromatography on controlled pore glass, carboxymethyl-Sephadex, and phosphocellulose (*6*). These steps result in three fractions, each of which is enriched in a different size class of interferons. Each of these is further fractionated by chromatography on octyl-Sepharose and isoelectric focusing (*3*, *4*). At this stage the specific activities of interferons A, B, and C are about 1.6×10^9 to 1.8×10^9 reference units per milligram of protein. The final step of purification is preparative polyacrylamide gel electrophoresis in the presence of SDS (*7*). This serves to improve the separation of the interferons A, B, and C from each other and to remove minor contaminating proteins, together with the ampholines remaining from isoelectric focusing.

The distributions of proteins and of interferon activity in the gel electropherograms of the preparations enriched in interferon class A, B, and C, respectively, are shown in Fig. 1. The interferon A preparation (Fig. 1A) gives rise to one major diffuse protein band (35,000 to 40,000 daltons) which comigrates with interferon activity. The specific activity of this preparation does not decrease during the gel electrophoresis.

The interferon B preparation (Fig. 1B) gives rise to a broad and diffuse band (25,000 to 40,000 daltons). The most heavily stained portion coincides with the 26,000- to 30,000-dalton region, whereas most of the interferon activity migrates in the 29,000- to 35,000-dalton region. This apparent discrepancy might

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Mouse interferons A and B

1 5

H-Ile-Asn-Tyr-Lys-Gln-Leu-Gln-Leu-Gln-

10 15

Glu-Arg-Thr-Asn-Ile-Arg-Lys-(?)-Gln-Glu-

20

Leu-Leu-Glu-Gln-Leu-

Mouse interferon C

1 5

H-Ala-Asp-Leu-Pro-Gln-Thr-Tyr- Asn-Leu-

10 15 20

Gly-Asn-Lys-Gly-Ala-Leu-Lys-Val-Leu-Ala-Gln

Fig. 2. Amino terminal amino acid sequences of mouse interferons A, B, and C. Abbreviations for the amino acid residues are: Ala, alanine; Asn, asparagine; Asp, aspartic acid; Arg, arginine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Pro, proline; Phe, phenylalanine; Ser, serine; Thr, threonine; Tyr, tyrosine; and Val, valine.

a

Mouse interferons A and B

H-Ile-Asn-Tyr-Lys-Gln-Leu-Gln-Leu-

Gln-Glu-Arg-Thr-Asn-

Human fibroblast interferon

H-Met-Ser-Tyr-Asn-Leu-Leu-Gly-Phe-

Leu-Gln-Arg-Ser-Ser-

b

Mouse interferon C

H-Ala-Asp-Leu-Pro-Gln-Thr-Tyr-Asn-Leu-Gly-Asn-

Lys-Gly-Ala-Leu-Lys-Val-Leu-Ala-Gln-

Human lymphoblastoid interferon

H-Ser-Asp-Leu-Pro-Gln-Thr-His-Ser-Leu-Gly-Asn-

Arg-Arg-Ala-Leu-Ile-Leu-Leu-Ala-Gln-

Fig. 3. Comparison of the amino terminal amino acid sequences of mouse interferons A and B with that of a human fibroblast interferon and of mouse interferon C with that of a human lymphoblastoid interferon. Identical amino acids in the same relative positions are underlined.

be caused by a differential inactivation of the interferons in the lower molecular size region during gel electrophoresis and elution. Indeed, the specific activity of the preparation decreases by 85 to 90 percent in the course of these procedures.

The interferon C preparation (Fig. 1C) gives rise to one heavy band (20,000 daltons) which comigrates with interferon activity and a minor contaminant (18,000 daltons). Well separated from these, a second interferon active region appears. This region reveals no stainable protein and corresponds in position to interferons A and B. These puzzling findings might be accounted for by the fact that the specific activity of interferon C decreases by more than 90 percent during gel electrophoresis and elution, whereas that of interferon A, as noted above, remains unchanged.

Proteins were eluted (*6*) from the following regions of the gel electropherograms: the shaded region (in Fig. 1A) in which the bulk of the protein and interferon activity comigrate (region I); the shaded region (b_1 in Fig. 1B) with most of the interferon activity (region II); the region with the bulk of the protein (b_2 in Fig. 1B) (region III); the shaded region (in Fig. 1C) in which interferon activity and the bulk of the protein comigrate (region IV).

The NH_2-terminal amino acid sequences of the eluted proteins were determined (*5*) (Fig. 2). There is an identity in the sequence of the first 24 amino acids (with the exception of No. 17, which has not been identified) among interferons eluted from regions I, II, and III, that is, between interferons A and B. The sequence of the first 20 amino acids from the protein in region IV (that is, interferon C) differs from that of interferons A and B in all but two positions (*8*).

The broad size distribution of the interferons designated as A and B is unexplained and so is the difference between these interferon species. These could be accounted for probably by one or more of the following phenomena: nonuniform glycosylation, nonuniform proteolytic cleavage, or even differences in amino acid sequence in portions of the molecule other than the NH_2-terminal region. In the case of interferons A and B, the identity of the NH_2-terminal sequences is in line with the similarity in tryptic peptide patterns (*4*). Furthermore, the difference in NH_2-terminal sequence is in line with the difference in tryptic peptide patterns between interferons A (or B) and interferon C (*4*).

There is a remarkable homology in NH_2-terminal amino acid sequence between mouse interferon C (Fig. 3b) and a human lymphoblastoid interferon (*9*). Of the first 20 amino acids, 13 are identical. All of the seven amino acid replacements could be the consequence of single base substitutions in the appropriate codons. There is a lesser homology between mouse interferons A (or B) (Fig. 3a) and a human fibroblast interferon (*10*): three of the first 13 NH_2-terminal amino acids are identical (*11*).

In summary, with respect to the first 20 residues, the mouse interferons are not homologous. In this NH_2-terminal region, however, the mouse A (or B) interferon is homologous to a human fibroblast interferon, and the mouse C interferon is homologous to a human lymphoblastoid interferon. These observations are consistent with the hypothesis that these mouse interferons have evolved from at least two distinct interferon genes in the mammalian ancestor, but alternative explanations can be envisioned.

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6. Crude interferons were applied to a column of controlled pore glass (*4*) and were eluted first with 0.4*M* glycine-HCl buffer (*p*H 3.5), 0.5 percent (by volume) glycerol, 10 μM phenylmethylsulphonyl fluoride, 3.1 m*M* NaN_3 and then with the same buffer at *p*H 2. The material recovered at *p*H 3.5 was fractionated through chromatography on phosphocellulose and the fractions were analyzed by SDS polyacrylamide gel electrophoresis. The fraction that eluted with 400 m*M* sodium phosphate buffer (*p*H 6.2) was enriched in interferon A; the one eluted with 600 m*M* sodium phosphate buffer (*p*H 6.2) was enriched in interferon B. The material recovered from controlled pore glass at *p*H 2 was similarly fractionated on phosphocellulose and analyzed. The fraction eluted with 400 m*M* sodium phosphate buffer (*p*H 6.2) was enriched in interferon C. Each of these three fractions was separately purified further by chromatography on octyl-Sepharose and by isoelectric focusing. Subsequently, the interferon-active fractions were dialyzed extensively against 2 m*M* sodium phosphate buffer (*p*H 6.2), 2 percent (by volume) glycerol, 0.2 percent (weight to volume) SDS, and 10 μM phenylmethylsulphonyl fluoride and concentrated five- to tenfold by treatment with Aquacide IIA (Calbiochem). Each of the concentrated interferon fractions (1.7×10^8 units of preparation A, 5×10^7 units of preparation B, and 6×10^7 units of preparation C) was dissolved in 0.4 to 0.7 ml of SDS sample buffer (*6*), heated at 95°C for 1 minute, and divided into five portions (in the proportion of 24:24:24:24:4). Each of the portions was subjected to electrophoresis through a 15 percent polyacrylamide slab gel in SDS (20 mA; 6 hours). The gel was then stained with 0.25 percent Coomassie brilliant blue in 25 percent methanol, 7.5 percent acetic acid for 10 minutes, and destained in 20 percent methanol, 7.5 percent acetic acid overnight. To find the interferon-containing regions in the gels, the track (with the smaller portion) was cut transversely into 3-mm slides in the re-

gion of the gel where the interferon activity was expected. Each slice was crushed into small pieces with a plastic rod and extracted into 0.3 ml of extraction buffer (137 m*M* NaCl, 2.7 m*M* KCl, 8.1 m*M* Na_2HPO_4, 1.5 m*M* KH_2PO_4, 0.1 percent (weight to volume) SDS, 10 percent (by volume) glycerol, and 10 μM phenylmethylsulphonyl fluoride) at room temperature overnight. The eluates were clarified by centrifugation and assayed for interferon activity by the cytopathic effect assay (*3*). The interferon-containing regions were cut from the remaining tracks and eluted essentially under the conditions described by E. Lazarides [*J. Supramol. Struct.* **5**, 531 (1976)], except that the gel pieces were immersed in running buffer and covered with a small piece of tissue paper (Kimwipe, Kimberly-Clark) rather than embedded in polyacrylamide. Following electrophoretic elution, each sample was dialyzed extensively, first against 150 m*M* NaCl, 0.1 percent (weight to volume) SDS, then by repeated dialysis against 0.02 percent (weight to volume) SDS, and finally lyophilized; about 40 to 50 percent of the protein was recovered. The approximate amounts of protein used for sequencing were: 400 pmole of interferon A, 40 pmole of interferon b_1, 120 pmole of interferon b_2, and 130 pmole of interferon C (Fig. 1).

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Splanchnic Somatostatin: A Hormonal Regulator of Nutrient Homeostasis

Abstract. *Free (~ 1600 daltons) somatostatin-like immunoreactivity was identified in arterial plasma of dogs that had received a test meal. Neutralization of circulating somatostatin while the dogs were consuming a fatty meal increased the plasma concentrations of triglycerides, gastrin, pancreatic polypeptide, and insulin after the meal. It is concluded that, in the dog, somatostatin is a true hormone that regulates the movement of nutrients from the gut to the internal environment.*

The physiologic role of the somatostatin-containing D cells of the islets of Langerhans, stomach, and gut (*1*) is unknown. Juxtaposition of D cells to certain of the known target cells of somatostatin is consistent with a local or "paracrine" role for the tetradecapeptide (*2*), but it is not known if somatostatin has hormonal functions, that is, if it circulates in plasma in a biologically active form. In this report we present evidence that somatostatin is a true hormone (*3*) which, like two other islet cell hormones, insulin and glucagon, plays a role in the homeostasis of ingested nutrients.

Previously we reported that somatostatin-like immunoreactivity (SLI) of pancreatic and gastrointestinal origin increases in the venous plasma of dogs after they have consumed various meals (*4*); this would be expected for a hormone with the postulated homeostatic role. But gel filtration at *p*H 7.4 to 8.8 of venous plasma from fasted dogs and dogs that had just eaten a meal revealed no measurable SLI in the range (~ 1600 daltons) of somatostatin; rather, all SLI eluted in the ~ 150,000-dalton range (*5*). Although this ~ 150,000-dalton SLI represents 1600-dalton SLI bound to a component of large molecular weight from which it can be dissociated at acidic *p*H (*5*), its biologic activity under physiologic conditions is uncertain. We therefore examined the arterial plasma of dogs to determine if any free, potentially biologically active SLI could be detected at *p*H 7.4 after they had eaten a meal. We applied 3-ml samples of pre- and postprandial arterial plasma obtained from five normal dogs to a jacketed Biogel P-6 column (1.6 by 35 cm) equilibrated at 37°C with a buffer (*p*H 7.4) containing 0.9 percent NaCl, 0.1*M* EDTA (ethylenediaminetetraacetic acid), 0.5 percent bovine serum albumin, 0.02 NaN_3, and 100 kallikrein inactivator units of aprotinin per milliliter. We measured the SLI in 2.7-ml fractions by a modification (*6*) of previously described methods (*7*) using an antibody (80C) directed against the central portion of the somatostatin. In arterial plasma from fasting dogs we detected no SLI in fractions coeluting with the somatostatin marker. However, in arterial plasma obtained from five dogs that had received an intragastric protein-acid load (250 ml of liver, followed by 200 ml of 1*N* HCl), which stimulated plasma SLI to 606 ± 112 pg/ml (mean ± S.E.M.; $N = 5$), we recovered 352 ± 58 pg of SLI per milliliter in the 1600-dalton zone. To our knowledge this is the first demonstration at physiologic *p*H of free endogenous SLI in post-hepatic plasma. We observed higher concentrations of free endogenous SLI in the portal vein plasma (*8*).

If, as we have proposed (*9*), a meal-stimulated increase in plasma somatostatin restrains the rate at which ingested nutrients enter the circulation, a somatostatin-deficient state should be accompanied by increased concentrations of nutrients in the circulation after meals. Therefore, we induced by passive immunization a somatostatin deficiency in a group of eight fasting dogs weighing from 10 to 12.5 kg. Fifteen minutes before a meal of 600 g of meat (60 g of fat and 60 g of protein) and again 60 and 120 minutes after the meal, we injected via a leg vein 20 ml of either nonimmune sheep serum as a control, or, 2 days later, 20 ml of sheep antiserum to somatostatin (*10*). We measured the concentrations of triglycerides in venous plasma by the meth-

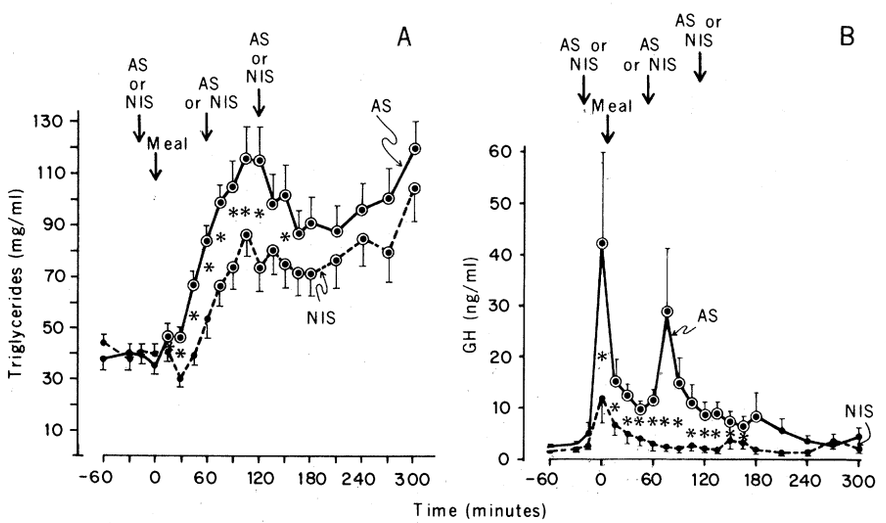

Fig. 1. (A) Comparison of the effect on plasma triglyceride levels of 20 ml of antiserum to somatostatin (*AS*) or of nonimmune sheep serum (*NIS*) injected 30 minutes before and 60 and 120 minutes after the ingestion of a fat-protein meal. Statistically significant ($P < .01$) differences are indicated by asterisks. (B) Measurements of canine growth hormone made during the foregoing experiments as evidence of neutralization of a biological action of circulating somatostatin.

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