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Unusual apparently constitutive interferons and antagonists in human placental blood

(α and β interferon subtypes/sarcolectin/pregnancy)

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ABSTRACT **We have detected seemingly uninduced interferons (IFNs) in 29/37 human placental samples obtained during caesarian sections at different periods of pregnancy, mostly around the 37th week. The amounts were usually low and did not enable us to correlate our findings with any physiological or pathological conditions. Occasionally the presence of IFN was masked by a lectin-like antagonist. Therefore, in a number of cases, substantially higher amounts of IFN were found after purification by affinity chromatography using concanavalin A, Cibacron blue, or antiserum to IFN-α, each coupled to Sepharose. Analysis by sodium dodecyl sulfate/polyacrylamide gel electrophoresis revealed the presence of IFN-α and IFN-β with molecular masses between 15 and 80 kilodaltons. Some of the high molecular weight components were neutralized either only by monospecific antiserum to IFN-α or, to the same extent, by antiserum to IFN-α or to IFN-β, reminiscent of those previously reported after viral induction in the human amniotic membrane. We postulate that both IFNs and antagonist play a physiological role during fetal development.**

Numerous recent publications suggest that interferon (IFN) is involved in fetal development at a yet-undetermined period and step (1, 2). Three arguments are in favor of this hypothesis. First, IFN-α can be constantly detected in human amniotic fluid at periods from 16 weeks of pregnancy on (3). Second, IFNs induced by viruses in the amniotic membrane have molecular sizes and antigenic constitutions apparently different from those found in adult tissues or plasma (4). Third, IFNs seem to play a role in the development of the cytoskeleton (1) and extracellular matrix of normal (5) and transformed (6, 7) cells.

These observations prompted us to search for and analyze uninduced IFNs excreted in human placentas or amniotic membranes. The results reported here show, indeed, that such IFNs can be constantly found in the majority of placental blood samples; however, these IFNs are often masked by the presence of IFN-antagonistic substances. One of these products was identified as being similar to or closely related to sarcolectins previously detected in hamster (8) and human (9) sarcomas and muscles.

MATERIALS AND METHODS

Chemicals. Bovine serum albumin (fraction V) was obtained from Calbiochem, pepsin (twice crystallized, 2.675 units/mg) from Worthington, ammonium sulfate and ethylene glycol from Merck. Methyl α-D-mannoside (grade III), 2-mercaptoethanol, and 1,4-dithiothreitol were supplied by Sigma Chemical Co.

Chromatographic Products. Sephadex G-25 medium in pre-packed PD 10 columns, concanavalin A (Con A)-Sepharose and Blue Sepharose CL-6B (group-specific adsorbents), cyanogen bromide-activated Sepharose 4B, and activated CH-Sepharose 4B (coupling gels for antiserum immobilization), and acrylamide were purchased from Pharmacia. The standard proteins used for calibration of the slab gels were also from Pharmacia.

Biological Preparations. Human placentas were collected shortly after caesarian section between the 37th week (calculated on the basis of amenorrhea and sonar exploration) and delivery. Most of the women were in the 39th week, corresponding to a fetal age of 37 weeks. In two additional cases, products of therapeutic abortion by vacuum aspiration in the 10th and 17th weeks of development were studied.

Fetal membranes were separated from the placenta, washed carefully one to five times in 500 ml of saline (0.15 M NaCl, pH 7), then incubated with Eagle's minimal essential medium (MEM), supplemented with 10% heat-inactivated newborn calf serum, at 37°C for 18 hr.

Blood from the umbilical cord was collected.

The whole placenta was then compressed for 3 hr under 0.15 kg/cm^2 (15 kPa) to squeeze out the blood, which was analyzed.

Cells and Viruses. Human diploid fibroblasts (F7000, Flow Laboratories) were grown in Eagle's basal medium (BME) supplemented with nonessential amino acids, 2 mM glutamine, and 10% heat-inactivated fetal calf serum. Human amnion (WISH), bovine kidney (MDBK), and mouse L929 cells, in continuous cultivation, and rat fibroblasts (REF) in secondary subcultures, were grown in Eagle's MEM supplemented with 10% heat-inactivated newborn calf serum. Normal rat kidney (NRK) cells were grown in RPMI 1629 medium, supplemented with 10% fetal calf serum and 4 mM glutamine.

Vesicular stomatitis virus (VSV), Indiana strain, and encephalomyocarditis (EMC) virus were grown on L929 cells and titrated by plaque-forming unit assay. EMC virus titer was also evaluated by human erythrocyte (O+ group) agglutination.

Affinity Chromatography on Con A-Sepharose. IFN preparations were applied to a Sephadex G-25 M PD 10 column (1.5 × 5 cm), previously equilibrated with 0.02 M sodium phosphate, pH 7.2/1 M NaCl (buffer E0) and eluted with the same buffer. The breakthrough active fractions (5 ml) were applied on 5 ml of Con A-Sepharose in a column (IBF 11, 1.14 × 10 cm) equilibrated with buffer E0 at room temperature at a flow rate of 4.5 ml/hr, according to a previously described procedure (10, 11). After 1-hr contact and washing with E0 buffer, a sequential elution was performed with 0.1 M methyl α-D-mannoside in the E0 buffer, called buffer E1, then with buffer E1 containing 50% (vol/vol) ethylene glycol, called

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Abbreviations: IFN, interferon; Con A, concanavalin A; VSV, vesicular stomatitis virus; EMC, encephalomyocarditis virus; IU, international reference units.

buffer E2. The bound substances were eluted at a flow rate of 20 ml/hr. Fractions (1 ml) were collected in 0.5 ml of a solution of 1% bovine serum albumin in 0.02 M sodium phosphate, pH 7.4, in glass tubes.

Affinity Chromatography on Immobilized Cibacron Blue F3G-A (Blue Sepharose CL-6B). After buffer exchange with Sephadex G-25 M, as described above, samples (3–4 ml) were applied to 3.5-ml Blue Sepharose CL-6B gel (12) in an IBF-11 column (1.14 × 10 cm), equilibrated with starting buffer (E0), and recycled continuously overnight at a flow rate of 4.5 ml/hr. After washing, the bound substances were eluted at a flow rate of 20 ml/hr by increasing the ionic strength of the buffer to 2 M NaCl (buffer E1'). Finally, residual material was eluted with 50% (vol/vol) ethylene glycol in buffer E0 (buffer E2').

Affinity Chromatography Using Immobilized Immune Serum to IFN-α. This procedure was based on methods previously described (13, 14). IFN-α antiserum was produced, according to the technique of Pyhälä (15), by immunizing sheep with 10^7 units of Sendai virus-induced human leukocyte IFN with a specific activity of 10^7 international reference units (IU)/mg of protein. Sheep antisera had complement inactivated by heating at 56°C for 30 min and gamma globulins were precipitated by ammonium sulfate (50%). Antisera were extensively absorbed against impurities (consisting of normal buffy coat, allantoic fluid, human serum and human albumin, and Sendai virus) and titrated before binding to cyanogen bromide-activated Sepharose 4B. One sample of each batch was tested for its capacity to retain IFN-α.

Placental blood IFNs were applied on previously unused Sepharose-coupled IFN-α antiserum (2.5 ml) in columns (Amicon Wright DC, 7.6 cm), equilibrated with phosphate-buffered saline, pH 7.2.

Crude IFNs were loaded onto the column at a flow rate of 4 ml/hr at 4°C and recycled overnight. After extensive washing with phosphate-buffered saline, bound material was eluted at pH 2.2, using citrate/phosphate buffer, 0.05 M (16), at a flow rate of 20 ml/hr.

Elution was achieved by adding 50% (vol/vol) ethylene glycol in citrate/phosphate buffer, pH 2.2. Then antibody-Sepharose was treated with 100 ml of 4 M guanidine and rinsed with 300 ml of phosphate-buffered saline.

NaDodSO$_4$/PAGE. Discontinuous polyacrylamide gel electrophoresis was performed in slab gels, according to Laemmli (17) and King and Laemmli (18), with a 5% acrylamide stacking gel and a 15% (wt/vol) separating gel. Fractions from different chromatographies were concentrated by lyophilization after dialysis against 0.03 M ammonium bicarbonate, then heated to 100°C for 2½ min in the presence of 2% NaDodSO$_4$ and 0.1 M 1,4-dithiothreitol in 0.05 M Tris·HCl, pH 6.8/10% (vol/vol) glycerol containing 0.002% bromophenol blue as the marker dye. Electrophoresis was carried out at 20 mA for 4 hr at room temperature. The amount of IFN applied to the gel varied between 3×10^3 to 3×10^4 IU. The protein concentration of samples applied to the gel varied from 0.5 to 1 mg of protein per ml, corresponding to 20–40 μg of protein in each well. One part of the gel was cut into tracks, which were sliced into 2-mm pieces and eluted for IFN assay. The other part of the gel was stained with 0.25% Coomassie brilliant blue. Gels were calibrated with the following protein standards: phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa).

Protein Concentration. Prior to the addition of NaDodSO$_4$ and Tris buffer, protein concentrations were estimated by absorbance at 280 nm or determined as described by Lowry *et al.* (19), using bovine serum albumin as standard.

IFN Assay. IFN activity was assayed by microtitration in previously grown cells, using the inhibition of VSV-induced cytopathic effect in two human cell lines, F7000 and WISH, or in rat and bovine cells. Titers were expressed in IU/ml. Induced human IFNs used for comparison were of leukocyte origin, with a specific activity of 10^{-6} IU/mg of protein. Mouse IFNs, prepared in C243 cells, were of the α or β type, with a specific activity of 10^{-6} IU/mg of protein after induction with Newcastle disease virus.

IFN Neutralization Procedure. Various crude and gel-purified IFN preparations were studied for the antigenic structures of their components by using antibodies raised against highly purified α and β IFNs (the latter was a generous gift of J. Vilcek). The assays were performed by chessboard titrations. The antibody titer was estimated, as suggested by Kawade (20), as the concentration of antiserum that reduced 10 IFN IU to 1.

Assay and Purification of an IFN Antagonist. The purification was that of Jiang *et al.* (8). First, the protein precipitating from crude placental blood at 25–70% saturation with ammonium sulfate was isolated and assayed for the capacity to agglutinate Rous sarcoma virus-transformed 3T3 rat cells in a microplate with V-shaped wells. The preparation was then treated with pepsin (Worthington, twice crystallized) at 2.675 units/mg and 1 mg/ml, in pH 2 buffer. The reaction mixture was incubated overnight at 37°C, and the reaction was stopped by adjusting the pH to 8 with NaOH. The pepsin-treated material was then filtered through Sephadex G-200 and assayed for cell agglutinating activity or dialyzed with the usual buffer for NaDodSO$_4$/PAGE.

The cell agglutination titer was determined as the dilution that agglutinated about 50% of the cells and was usually around 128 units.

RESULTS

Presence of Spontaneously Released IFN in Human Placental Blood and Human Amniotic Membranes. We looked for IFN in placental blood, the amniotic membrane, and umbilical cord blood. The specimens were assayed in human F7000 and occasionally in WISH, REF, NRK, and MDBK cells. As shown in Table 1, IFN was found in 29/37 placental samples tested in F7000 and WISH cells. Rat cells were also sensitive, while MDBK cells were rarely effective for the detection of IFN in the crude material (data not shown). In suspended human amniotic membrane, in the absence of any induction, IFN was found released in the tissue culture medium (14 positive out of 29 tested). Blood from the umbilical cord also contained small amounts of IFN.

As shown in Table 2, samples were obtained between the 35th and 38th week of pregnancy. Only two were taken before the 17th week and one after the 38th week. Both of the early specimens were negative. The IFN content was found to be independent of parameters, such as sex, body weight at birth, and weight of the placenta. Furthermore, pathological conditions, such as herpesvirus infection, Rh isoimmune risk, or fetal injury motivating caesarian section, were not found to

Table 1. IFN activity in samples from 37 caesarian sections

Sample	No. of positive samples at indicated IFN titer			Total positive samples
	10 IU/ml	10–60 IU/ml	≥100 IU/ml	
Placental blood	9	5	15	29/37 (78%)
Amniotic membrane	6	5	3	14/29 (48%)
Umbilical cord blood	1	1	1	3/10 (30%)

IFN was titrated in either F7000 or WISH cells, both human.

Table 2. Distribution of detectable amounts of IFN in placental blood samples

Age of fetus, weeks	No. of samples	No. of positive samples at indicated IFN titer			Total positive samples
		10 IU/ml	10–60 IU/ml	≥100 IU/ml	
10	1	—	—	—	0/1
17	1	—	—	—	0/1
35	6	2	1	2	5/6
36	4	1	1	1	3/4
37	19	4	2	10	16/19
38	5	2	1	1	4/5
40	1	—	1	—	1/1

interfere with the presence of IFN or to affect the amount detected (data not shown).

Purification and Identification of the IFNs Present in Placental Blood. To characterize the different IFNs, crude placental blood samples from different donors were analyzed by using three different chromatography procedures, Con A-Sepharose, Cibacron blue Sepharose, and a Sepharose affinity column with antibody to IFN-α.

The biologically active fractions obtained after filtration on Sephadex G-25 medium were applied to Con A-Sepharose. After washing with the starting buffer, we found one peak, containing IFN-α, that was not bound to the immobilized Con A (Fig. 1). A second peak appeared after elution with 10 mM methyl α-D-mannoside. A third peak was recovered after subsequent elution with both methyl α-D-mannoside and ethylene glycol. The IFNs found in the two latter peaks were identified by specific immune sera as being of the β type. The apparently increased IFN concentration seen after purification suggested the possible presence of antagonistic substances or inhibitors in the crude material.

After chromatography on Blue Sepharose, four peaks were found when the fractions were tested in MDBK cells. They were respectively called A, B, C, and D (Fig. 2). Peak A contained the breakthrough fractions, identified as IFN-α species, and the bulk of proteins. After elution with 2 M NaCl, a second peak, B, was found. A third peak, C, appeared that contained, in addition to IFN, a substance antagonistic to the antiviral effect, which will be analyzed further on. Buffer E2′ (ethylene glycol in 1 M NaCl buffer) eluted a fourth peak, D, consisting of IFNs neutralized to the same extent by either IFN-α or IFN-β immune sera (data not shown). To study their composition, peaks B, C, and D were thereafter submitted to NaDodSO$_4$/PAGE analysis as detailed further.

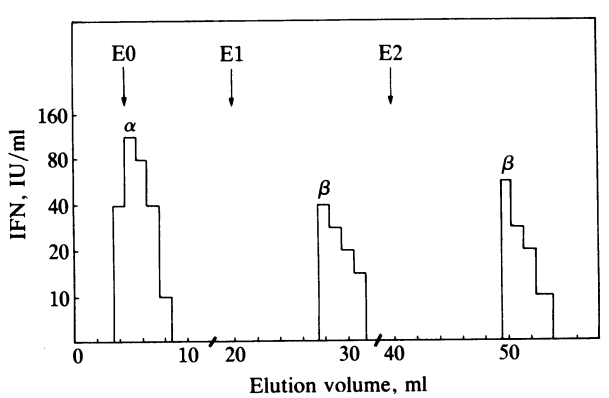

FIG. 1. IFNs from placental blood separated on Con A-Sepharose columns. After Sephadex G-25 filtration, a 5-ml IFN preparation (40 IU and 2.2 mg of protein per ml) was applied on Con A-Sepharose in E0 buffer. Material was eluted with buffers E1 and E2 as indicated. Biological activity was assayed with WISH cells.

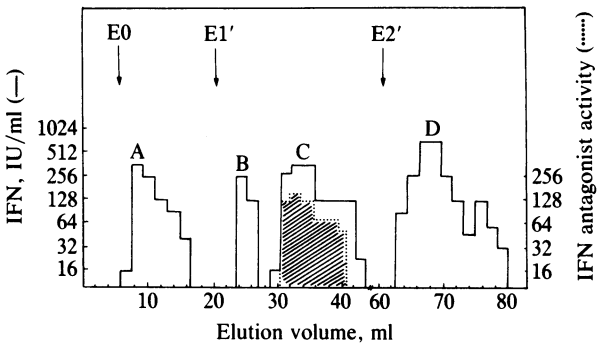

FIG. 2. IFNs from placental blood obtained after chromatography through Blue Sepharose. Fractions (5 ml with no antiviral activity) eluted from Sephadex G-25 M in buffer E0 were loaded on Blue Sepharose equilibrated with this starting buffer. After washing, elution was performed with buffer E1′ (which contains 2 M NaCl), then with buffer E2′ (which contains 50% ethylene glycol). Biological activity was measured on MDBK cells. The hatched area represents antagonist activity, where protection from VSV cytopathogenicity disappears.

Chromatography using specific immune sera to IFN-α was used to study various blood placental IFN preparations. The elution profile of a representative experiment is shown in Fig. 3. Almost all protein material eluted from the column was in the flow-through fractions, with a small amount of IFN that was neutralized by either anti-IFN-α or anti-IFN-β antibody to the same level, as defined in *Materials and Methods* (data not shown). A major peak, G, of IFN-α was found after detachment of the bound molecules from the ligand with pH 2.2 buffer.

Analysis of the Different IFN Peaks by NaDodSO$_4$/PAGE. Peaks B and D, obtained after Blue Sepharose chromatography, and the major peak, G, separated by the anti-IFN-α antibody column were further analyzed by using NaDodSO$_4$/PAGE under reducing conditions. The results obtained varied according to the purification procedure and also the cell used for the antiviral assay of the different chromatographic fractions, thus showing the heterogeneity of the molecular composition. As shown in Table 3, the IFNs could be grouped on the basis of their molecular weight. They were comparable to those previously found in the human amniotic membrane after induction (4). In peak B, isolated by Blue Sepharose chromatography, a 21- to 22-kDa component was neutralized only by IFN-α antiserum, while a 43-kDa component was neutralized by anti-IFN-α or anti-IFN-β to about

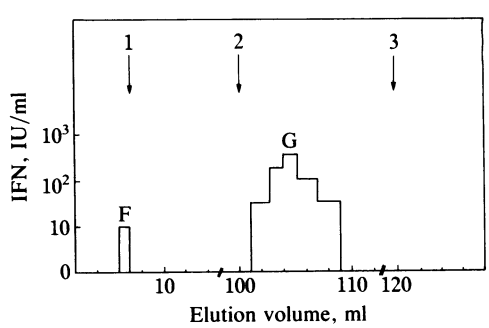

FIG. 3. Purification by antibody affinity chromatography. Placental blood (3 ml) was loaded on 2.5 ml of anti-IFN-α globulin-Sepharose and recycled overnight as described in *Materials and Methods*. The Sepharose column was sequentially rinsed with phosphate-buffered saline (arrow 1) and developed with 0.05 M citrate/phosphate buffer, pH 2.2 (arrow 2) and finally with 50% (vol/vol) ethylene glycol in citrate/phosphate buffer, pH 2.2 (arrow 3). MDBK cells were employed for titration.

Table 3. Analysis by $NaDodSO_4$/PAGE of placental IFNs purified and concentrated by Blue Sepharose or by immune affinity chromatography

Molecular mass of component, kDa	Assay cell type	IFN titer, IU		
		Blue Sepharose chromatography		Antibody to IFN-α chromatography
		Peak B	Peak D	Peak G
80	F7000	32	64	32
	MDBK	8	32–64	<8
43	F7000	32	32	256
	MDBK	8	<8	<8
26	F7000	64	<8	<8
	MDBK	16	<8	8–16
21–22	F7000	128	32	<8
	MDBK	128	16–32	<8
15–17	F7000	8	<8	128
	MDBK	32	<8	8

Peak fractions as indicated in Figs. 2 and 3.

the same titer. A 80-kDa molecule, although stable under denaturing conditions, was difficult to identify, perhaps because of its capacity to adsorb to different surfaces. In peak D, a 21- to 22-kDa component was found to be neutralized by IFN-α immune serum and an 80-kDa form was partially neutralized by anti-IFN-α.

In peak G, the IFNs retained by anti-IFN-α immune adsorbent contained by definition IFN-α antigenic determinants. Their molecular masses were 80 kDa (in small amounts), 43 and 26 kDa (in small amounts), and 15–17 kDa.

To eliminate the possibility of aggregates persisting after heating, $NaDodSO_4$, and reducing conditions, we performed additional control experiments. Small amounts of IFN-α were added to placental tissue extracts containing 40 μg of protein per well and these mixtures were then subjected to the same denaturing treatment and $NaDodSO_4$/PAGE analysis. The results (not shown here) clearly indicated that no IFN aggregates were produced. Furthermore, the mixture of reference proteins at the same or higher concentrations separated correctly.

Presence of an IFN Antagonist in Placental Blood. In a number of experiments, IFN could not be detected directly from placental blood, or had a low titer, but appeared clearly after all of the purification procedures described. In 18/37 samples, the presence of an IFN antagonistic substance was suspected because of the virus inhibitory action that appeared only at a higher dilution of the crude mixture and was thereafter proportional to the dilution. This zonal effect was also found in a chromatographic fraction (Fig. 2, peak C, hatched area).

Since an IFN antagonist from hamster sarcoma and muscle was recently described and identified (8), it was tempting to analyze the antagonist detected here on the basis of known biological and physicochemical characteristics. The purified preparations, as indicated in *Materials and Methods*, were assayed as follows for IFN-antagonistic activity. Mouse L cells were incubated with 500 reference units of mouse IFN-α/β. The choice of this cell species was motivated by the known heterospecific crossings of the lectin-like antagonist. After 5 hr, IFN was removed and 1:10 and 1:20 dilutions of antagonist were added and further incubated for 18 hr at 37°C. Then the cells were thoroughly washed and EMC virus was added for a further 18-hr incubation. The virus yields were estimated by hemagglutination (HA) of human O+ erythrocytes. In typical experiments, the HA titer of the virus control was 256, that of the IFN-treated cells was <2, and, in the presence of the antagonist, the virus titer increased to 64 HA units. These experiments could be routinely repeated.

Finally, physicochemical characteristics were analyzed in the peak C fraction obtained after Blue Sepharose chromatography (Fig. 2). The fractions (30–36 ml effluent volume) were concentrated 8-fold by lyophilization and treated or not by pepsin (1 mg/ml) at 37°C, pH 2, for 20 hr. The enzymatic reaction was stopped by adjusting the pH to 7 with 0.1 M Tris·HCl. As a reference, bovine serum albumin (0.5 and 1 mg/ml) was employed. The protein samples were heated at 100°C for 2½ min in the presence of 2% $NaDodSO_4$ and 0.1 M 1,4-dithiothreitol and analyzed by electrophoresis. The IFN antagonist migrated in the 60- to 65-kDa region (Fig. 4, lane 5). The antagonist was unaltered by a 20-hr pepsin treatment (lane 6), while the control albumin (lanes 1 and 3) was completely destroyed (lanes 2 and 4). As published previously, the excised band contained both cell-agglutinating and antagonistic activities (8).

DISCUSSION

The results reported here suggest that the uninduced human IFNs regularly found in the amniotic fluid derive from the placenta, since IFN is detected with a higher frequency in this site (78% against 48% in the human amniotic membrane and 30% in the umbilical cord). The titers vary from 10 to 100 IU/ml, with occasional peaks reaching 1000 IU/ml. We were unable to correlate the amount of IFNs present either with the developmental status of the pregnancy or with other physiological or pathological conditions.

In some experiments, the presence of IFNs can be masked by antagonistic substances that at low dilutions are able to block the antiviral effect during titration. Since there is no direct relationship between IFN and the antagonistic molecules, their separation results in a complete expression of protection from virus. Furthermore, in a number of experiments no IFN was found in the crude blood extracts, while

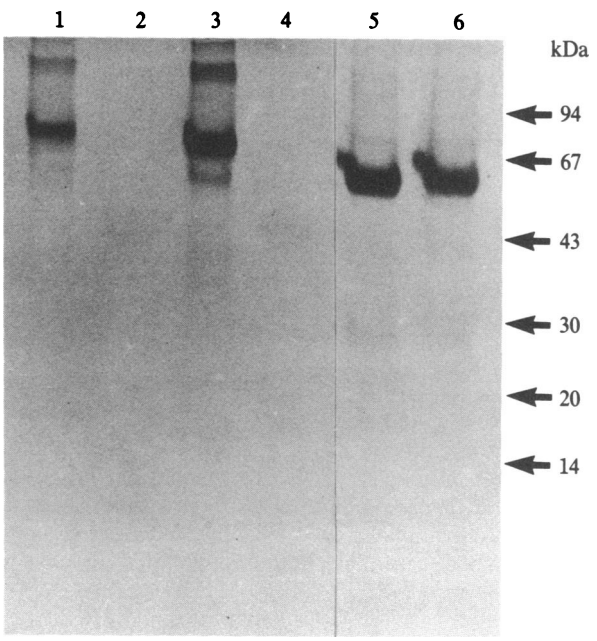

FIG. 4. $NaDodSO_4$/PAGE analysis of the purified IFN antagonistic substance, treated or not by pepsin. Bovine serum albumin, as control, was loaded in lanes 1–4. Lane 1, albumin at 500 μg/ml; lane 2, albumin at 500 μg/ml, pepsin treatment; lane 3, albumin at 1 mg/ml; lane 4, albumin at 1 mg/ml, pepsin treatment. The IFN antagonistic substance detected in peak C after Blue Sepharose chromatography is illustrated in lanes 5 and 6: Lane 5, prior to pepsin treatment; lane 6, after pepsin treatment.

significant amounts were retained thereafter by affinity chromatography or other separation methods. In addition, from some fractions during Cibacron blue Sepharose purification, an interferon antagonist was detected that promotes the decay of protection from virus established by IFN in the cell. This substance is resistant to pepsin, heat, and $NaDodSO_4$, agglutinates mouse cells, and migrates in a $NaDodSO_4$ gel in the 60- to 65-kDa region, reminiscent of "sarcolectin" isolated from hamster tumors (8).

Some of the IFNs detected in the placental blood are comparable to those found in the amniotic membrane after induction. The smaller components, 15–17, 21–22, and 26 kDa, seem to share antigenic properties with IFN-α. The 43-kDa component, which in the amniotic membrane is neutralized to the same extent by antibodies raised to either IFN-α or IFN-β, is also detected. This variant is equally retained by the IFN-α affinity column. One of the 80-kDa components is neutralized by antiserum to IFN-α, but to a lesser extent. It is presently unknown whether these unusual molecular species are products of genes that express themselves only during embryonic development or result from transcriptional or post-transcriptional procedures. Since they are detected under denaturing and reducing conditions, we can exclude the possibility that they are simple aggregates. This is also supported by adequate control experiments, as reported in *Results*. It is also unlikely (but cannot be excluded) that they are multimeric forms of a monomer, because in some cases the antigenic structure carries epitopes from both IFN-α and IFN-β, at an unusually high level. Because of the small amount of IFN excreted, further structural identification of the molecule cannot be obtained by biochemical or immunological methods. In addition, IFNs are now defined by the structure of the responsible gene and not by the product. Final characterization of constitutive human placental IFNs will therefore be based on recombinant DNA analysis.

The higher frequency of detection in placental samples suggests that IFN is not induced because of an accidental transplacental infection. Although virus-like particles have been reported in the human placenta (21), their presence is too inconstant to explain the regular detection of IFN in this site. Virions, morphologically characterized by electron microscopy, are of the retrovirus type, which usually does not induce IFN synthesis. Furthermore, no viruses or bacteria could be isolated from any second-trimester amniotic fluid samples (22).

Because of the known effects of IFN on the development of the cytoskeleton and extracellular matrix and its capacity to decrease cell growth (23), IFN could play a moderating but coordinated regulatory role in cell proliferation and increase the anchorage capacity of certain differentiating cells during fetal development. Indeed, it has been shown by Tomida *et al.* (24) that, in the presence of a differentiation-stimulating D factor, mouse myeloid leukemic M_1 cells can be induced to transform into macrophages or granulocytes. These authors postulate that although IFN is not responsible *per se* for differentiation, it promotes this process. Furthermore, the IFNs detected in animal placentas, either in mouse (25) or in rat (unpublished data), are found only after 10 days of gestation, when differentiation is already established. It can thus be suggested that IFNs could interfere only with fetal development.

Finally, these constitutive IFNs could be involved in the protection of fetuses against the immune reaction of the host, thus playing a role in immune tolerance.

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