INFECTION AND IMMUNITY, Jan. 1979, p. 80-86
0019-9567/79/01-0080/07$02.00/0

Vol. 23, No. 1

Large-Scale Production and Partial Purification of Mouse Immune Interferon

L. C. OSBORNE, J. A. GEORGIADES, AND H. M. JOHNSON*

University of Texas Medical Branch, Department of Microbiology, Galveston, Texas 77550

Received for publication 8 August 1978

Large-scale production of high-titered ($10^{2.2}$ to 10^4 U/ml) immune interferon (type II) was carried out in roller cultures of mouse spleen cells by using the T-cell mitogen staphylococcal enterotoxin A. Precipitation of 90% of this interferon by 55 to 80% saturated ammonium sulfate resulted in a 20-fold concentration and a two- to sixfold purification. After application of this interferon to either bovine serum albumin (BSA)-Affi-Gel 10 or hydroxylapatite columns, 100% of the interferon activity was recovered. By BSA-Affi-Gel 10 chromatography, 7% of the recovered activity was not bound, 45% was eluted with pH gradient 5 to 7, and 48% was eluted with 1 M NaCl. The pH- and salt-eluted interferons from the BSA-Affi-Gel 10 column were purified 62- and 390-fold, respectively, when compared with the starting materials. Rechromatography of the pH- and salt-eluted interferon peaks from the BSA-Affi-Gel 10 column did not alter their elution patterns. Stepwise elution of interferon from the BSA-Affi-Gel 10 columns with buffers of various pH and salt contents also resulted in greater than 300-fold purification. Specific activities of up to 2×10^5 U of interferon per mg of protein were attained with either elution procedure from BSA-Affi-Gel 10 columns. By hydroxylapatite chromatography, 5% of the recovered activity was not bound, 20% was eluted with a salt gradient, and 75% was eluted with 30% glycerin. Purification was 107- and 16-fold, respectively, for the two fractions. Ultrogel AcA 34 chromatography of the interferon resulted in two peaks of activity, a major one with a molecular weight of approximately 40,000 and a minor peak of molecular weight 70,000 to 90,000. Thus, by different types of chromatography, immune interferon was found to be heterogeneous.

Based on recent findings, the human and mouse interferon systems can be provisionally classified into two groups. These are the virus-type (type I) and immune (type II) interferons (9, 13). Virus-type interferons are classically induced by viruses or synthetic polynucleotides, whereas immune interferons are usually induced in primed lymphocytes by specific antigen or in unprimed lymphocytes by T-cell mitogens (11, 18, 26, 28, 31). Virus-type interferons which are stable at pH 2 are heterogeneous, and at least two antigenically distinct types exist (12, 23). They are called fibroblast and leukocyte interferons, indicating their cellular source. Immune interferon is labile at pH 2 and antigenically distinct from virus-type interferon (17, 32). The antigenic relationship of mitogen-induced and antigen-induced interferons is not known.

Considerable success has been obtained in the purification of mouse virus-type interferons produced by C-243 cells (7), by Ehrlich ascites tumor cells (19), and by L cells (6, 20, 25). Similar success has been achieved in purification of human leukocyte interferon (1, 15, 29, 30), human lymphoblastoid cell interferon (4), and human fibroblast interferon (1, 8, 15, 21, 29). In some cases, the interferons may have been purified to homogeneity (7, 19, 21).

The purification of immune interferon has not progressed as much as that of virus-type interferon. Purification of this interferon would be greatly facilitated if an inducer were available that stimulated large quantities in cell culture. Using the mouse system and a T-cell mitogen, we report here a system for large-scale production and partial purification of immune interferon. The ability to obtain high-titered, partially purified immune interferon provides a basis for definitively studying its biological properties as well as the production of specific antibodies.

MATERIALS AND METHODS

Protein determination. Protein concentrations were measured by fluorometric assay (3). Fluorescamine was obtained from Roche Diagnostics, and bovine serum albumin (BSA), obtained from Calbiochem, was used as a standard.

Interferon assay. Interferon was assayed on mouse L-929 cells as described previously (5) except that 0.5% methylcellulose was substituted for carboxymethylcellulose. Interferon activity is expressed in terms of the National Institutes of Health reference interferon. One unit of interferon is defined as the concentration that results in 50% plaque reduction of vesicular stomatitis virus (40 plaque-forming units per challenge dose).

SEA. Staphylococcal enterotoxin A (SEA) was produced and purified by the Microbial Biochemistry Branch, Division of Microbiology, Food and Drug Administration, Cincinnati, Ohio (2). SEA migrated as a single band on sodium dodecyl sulfate-polyacrylamide electrophoresis at a molecular weight of approximately 28,000 and eluted from a Sephadex G-75 column as a single, symmetrical peak.

Mice. All mice used in these studies were female C57Bl/6 mice (The Jackson Laboratory, Bar Harbor, Maine), 8 to 15 weeks old.

Large-scale production of immune interferon. A system was devised to make large quantities of mouse immune interferon. Spleens were removed from mice and teased apart in 2 to 5 ml of RPMI 1640 medium with forceps. Tissue debris was allowed to settle out in 50-ml conical tubes, and the dissociated cells were collected by centrifugation at 1,000 rpm in a Sorvall RC-3 centrifuge (HL-8 rotor) at 4°C. The cells were suspended to 3×10^6 to 5×10^6 cells per ml in RPMI medium. SEA, the immune interferon inducer, was added to a final concentration of 0.02 μg/ml. 2-Mercaptoethanol at a 10^{-5} M final concentration was added to facilitate continuous high yield of interferon (16). Fetal calf serum was added to a final concentration of 10%. The system was buffered with 0.015 M Tricine [*N*-tris(hydroxymethyl)methylglycine], pH 7.2 (Sigma Chemical Co., St. Louis, Mo.). Roller bottles (2,000 ml) were seeded with 200 ml of the cell suspension. The tightly capped bottles were placed on a roller apparatus and rotated at 8 rpm at 37°C for 3 days. The supernatant was collected after centrifugation at 2,000 rpm in a Sorvall RC-5 centrifuge (GSA head) at 4°C and stored at −70°C.

Concentration of immune interferon. Interferon was concentrated by ammonium sulfate precipitation. Crystalline ammonium sulfate was added to culture fluids to 55% saturation, and the precipitate was removed by centrifugation at 6,000 rpm (Sorvall RC-5 centrifuge, GSA head). The supernatant fraction was further precipitated by adding crystalline ammonium sulfate to 80% saturation. The percent saturation was based on the initial volume of the preparation. The entire precipitation procedure was carried out at 0°C in an ice bath. The precipitates were dissolved in distilled water and dialyzed exhaustively against phosphate-buffered saline (PBS; 0.15 M, pH 7.2) at 4°C. The non-precipitated materials were similarly dialyzed against PBS.

Removal of SEA inducer from concentrated immune interferon. SEA was specifically removed from the concentrated preparation of immune interferon by immunoabsorption. The Cowan strain of *Staphylococcus aureus*, which has a high concentration of protein A in its cell wall, was grown in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) under standard conditions at 37°C for 24 h. The cells were harvested by centrifugation at 10,000 rpm (Sorvall RC-5 centrifuge, GSA head) for 10 min, washed five times with PBS, suspended in PBS, heated at 80°C for 5 min, and stored at 4°C in 0.5% Formol-saline. Before use the stored cells were washed three times with PBS and packed by centrifugation. The protein A of the cells specifically bound immunoglobulin G by the Fc portion (10). A 1-ml amount of packed cells was mixed with 1 ml of rabbit hyperimmune antisera to SEA (R. Bennett, Food and Drug Administration), and the mixture was incubated at 4°C for 3 h. The *Staphylococcus*-anti-SEA complex was washed three times with PBS and incubated at 4°C for 24 h with concentrated (20×) immune interferon at a 1:10 volume ratio of adsorbent to interferon. The adsorption was monitored by the addition of trace amounts of ^{125}I-labeled SEA to the concentrated interferon preparation. Radioactivity was measured on a Nuclear Chicago model 1185 gamma scintillation counter.

Gel filtration of mouse immune interferon on Ultrogel AcA 34. An Ultrogel (LKB Instruments Inc., Rockville, Md.) column (2.5 by 60 cm) was equilibrated with PBS, and UV absorbance was monitored at 280 nm (ISCO model UA-5 absorbance monitor). The column was standardized with known-molecular-weight substances (blue dextran, BSA, ovalbumin, and myoglobin). Interferon which had been exhaustively dialyzed against PBS at 4°C was loaded on the column and pumped through at a rate of 10 to 15 ml/h. Five-milliliter fractions were collected.

Hydroxylapatite chromatography. Hydroxylapatite chromatography was performed by modification of a previously described technique (14). Briefly, commercially prepared hydroxylapatite (lot 15377; Bio-Rad Laboratories, Richmond, Calif.) was packed in a column (0.9 by 8 cm). Interferon (6 to 10 ml) was dialyzed against 0.02 M sodium phosphate, pH 7.0, and loaded onto the column. The column was then washed with the same buffer, eluted with a linear NaCl gradient (0 to 3 M), and then eluted with 30% glycerin (Fischer Scientific Co., Pittsburgh, Pa.) in 3 M NaCl. Fractions of 2 ml were collected. The relative amount of NaCl in each fraction during the NaCl gradient was evaluated by testing the conductivity of the fractions with a conductivity meter (model CDM3; The London Co.).

BSA-Affi-Gel 10 affinity chromatography. For these studies, we modified a previously described method (6). Purified BSA (Nutritional Biochemicals Corp., Cleveland, Ohio) was linked to Affi-Gel 10 (Bio-Rad) according to the directions of the manufacturer. Briefly, 25 ml of a solution of BSA (15 mg/ml in 0.1 M sodium phosphate, pH 7.0) was added to a vial containing 15 g of lyophilized Affi-Gel 10, and the mixture was stirred overnight at 4°C. A column (0.9 by 5 cm) was packed with this material and washed with 1 M NaCl, and the UV adsorbance at 280 nm of the effluent was monitored. The interferon was dialyzed against 0.05 M sodium acetate (pH 5.0) for 16 h, and samples (7 to 12 ml) were loaded onto columns which had been equilibrated with 0.05 M sodium acetate (pH 5.0). The columns were washed with the sodium acetate buffer and eluted with a pH gradient (pH 5 to 7; the acetate

solution was mixed with 0.02 M sodium phosphate, pH 7.0, in a gradient maker), followed by 1 M NaCl in 0.02 M sodium phosphate, pH 7.0. Fractions of 2 ml were collected.

RESULTS

Large-scale production and concentration of immune interferon. Interferon yields from pooled roller bottle cultures of spleen cells varied from $10^{2.7}$ to 10^4 U/ml. The results of ammonium sulfate precipitation of a representative immune interferon preparation are presented in Table 1. Sequential precipitation with 55 and 80% saturated ammonium sulfate resulted in recovery of over 95% of the immune interferon in the 80% precipitate. The procedure resulted in a 20-fold concentration and a twofold purification. When experiments were repeated, the purification ranged from two- to sixfold.

Under small-scale conditions, SEA was found to be a better inducer of immune interferon in C57Bl/6 mouse spleen cell cultures than either concanavalin A or phytohemagglutinin P (18). These three inducers were compared for their relative abilities to induce immune interferon under large-scale culture conditions, and, as previously observed, SEA was the best inducer (data not shown). Conditions of culture were varied to determine the best pH of incubation, length of incubation, and concentration of cells and SEA for optimal interferon production. The variations in these conditions were as follows: pH, 6.8 to 7.8; length of incubation, 12 h to 4 days; concentration of cells, 5×10^5 to 2×10^7 cells per ml; and concentration of SEA, 0.002 to 1.0 μg of SEA per ml. Interferon was produced optimally when cultures were grown at a pH of 7.2 for 3 days with a concentration of 3×10^6 to 5×10^6 cells per ml. The optimal concentration of SEA was 0.02 μg/ml.

^{125}I-labeled SEA absorption data from a representative interferon concentrate are given in Table 2. The immunoadsorbent was highly effective in removing essentially all SEA while not affecting the immune interferon titer. Concentrated interferon, with SEA removed, was used for all subsequent experiments.

Gel filtration. Ultrogel AcA 34 chromatography data on the heterogeneity and apparent molecular weight of an immune interferon preparation are presented in Fig. 1. Interferon, 10,000 U in 5 ml (specific activity, 62.5 U/mg of protein), was applied to the column, and 67% of that was recovered. More than 90% of this was found in fractions corresponding to a molecular weight of approximately 40,000. The remainder was eluted in the molecular weight range of 70,000 to 90,000. Fraction 29, the peak of interferon activity, had a specific activity of 17,500 U/mg of protein, corresponding to a 281-fold purification over crude interferon. The pool of fractions 28 to 31 had a specific activity of 7,944 U/mg of protein, indicating a 209-fold purification.

Hydroxylapatite chromatography. The elution profile from a hydroxylapatite column is presented in Fig. 2. Approximately 65,000 U of interferon in 6.5 ml (specific activity, 256 U/mg of protein) was applied to the column, and recovery was 114%. Most of the interferon, as well as the protein, was bound to the column; only 5% of the recovered interferon was in the breakthrough fractions. The interferon eluted by salt and glycerin represented 20 and 75%, respectively, of the recovered interferon. Fraction 38 of the salt gradient had a specific activity of 27,300 U/mg of protein, which corresponded to 107-fold purification. Fraction 61 of the glycerin-eluted peak had a specific activity of 4,100 U/mg of protein, corresponding to 16-fold purification.

TABLE 2. *Adsorption of ^{125}I-labeled SEA from immune interferon by anti-SEA antibody bound to S. aureus*[a]

Anti-SEA adsorbent treatment	Interferon preparation		
	SEA		Interferon (U/ml)
	cpm	μg/ml	
Starting material	11,676	4	3,000
Bound material	10,412	4	NT[b]
Present in absorbed supernatant	9	<0.003	4,000

[a] Interferon preparation was absorbed as described in the text with anti-SEA immunoglobulin G that was complexed to the Cowan strain of *S. aureus*.

[b] NT, Not tested.

TABLE 1. *Concentration of immune interferon by differential ammonium sulfate precipitation*

Fraction	Concn (U/ml)	Vol (ml)	Total no. of units	Sp act (U/mg)
Starting material	2,200	1,100	2,420,000	690
55% Ammonium sulfate precipitate	2,000	55	120,000 (5%)[a]	130
80% Ammonium sulfate precipitate[b]	40,000	55	2,200,000 (91%)	1,510
80% Ammonium sulfate soluble	10	1,100	11,000 (0.5%)	17

[a] The numbers in parentheses are the percentages of the starting material recovered in a given fraction.

[b] The precipitation with 80% ammonium sulfate represents sequential precipitation at 55 and 80% ammonium sulfate.

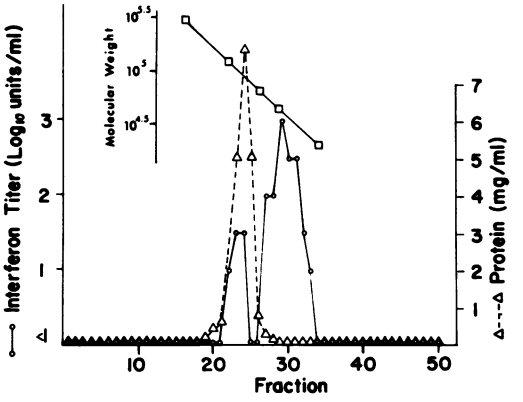

FIG. 1. *Filtration of mouse immune interferon by Ultrogel AcA 34 column chromatography. The column was loaded with interferon (10,000 U in 5 ml) and washed with PBS. The molecular weight range of this column was 20,000 to 350,000. Molecular weight standards were: blue dextran, >350,000; BSA dimer, 134,000; BSA, 67,000; ovalbumin, 45,000; and myoglobin, <20,000.*

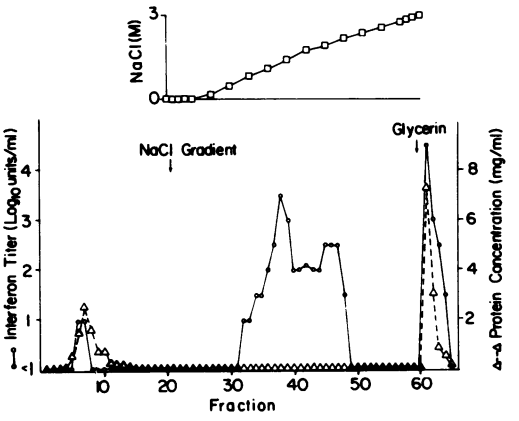

FIG. 2. *Fractionation of mouse immune interferon by hydroxylapatite chromatography. The column was loaded with 65,000 U of interferon in 6.5 ml and washed with 0.02 M sodium phosphate, pH 7. The column was then eluted with an NaCl gradient (0 to 3 M), followed by 30% glycerin, as indicated on the figure.*

Because interferon was eluted by both salt gradient and glycerin, these data provide evidence for possible heterogeneity in the immune interferon preparation.

BSA-Affi-Gel 10 chromatography. The elution profile from this column is illustrated in Fig. 3. Approximately 85,000 U of interferon in 8.5 ml (specific activity, 256 U/mg of protein) was applied to the column, and recovery was 156%. Most of the interferon was bound to this column, but most of the protein was not; only 7% of the recovered interferon was in the breakthrough fractions. The pH shift and the salt elution steps accounted for 45 and 48%, respectively, of the recovered interferon. The specific activity of fraction 38 (eluted during the pH shift) was 65,000 U/mg of protein, which corresponded to a 250-fold purification. The average specific activity of the pH-eluted interferon (fractions 30 to 43) was 16,000 U/mg; the purification was 62-fold. The average specific activity of the salt-eluted interferon (fractions 46 to 53) was 15,000 U/mg, or a 60-fold purification. Fraction 51, the peak fraction of interferon activity, was purified 390-fold, with a specific activity of 100,000 U/mg of protein.

Three peaks of interferon activity were also obtained when lesser amounts and different preparations were similarly chromatographed. However, when Affi-Gel 10 obtained from the manufacturer was in an alcohol suspension rather than in lyophilized form, almost no interferon (<1%) was bound to the column.

Rechromatography of interferon from BSA-Affi-Gel 10 columns. We did an experiment to determine whether pH- and salt-eluted

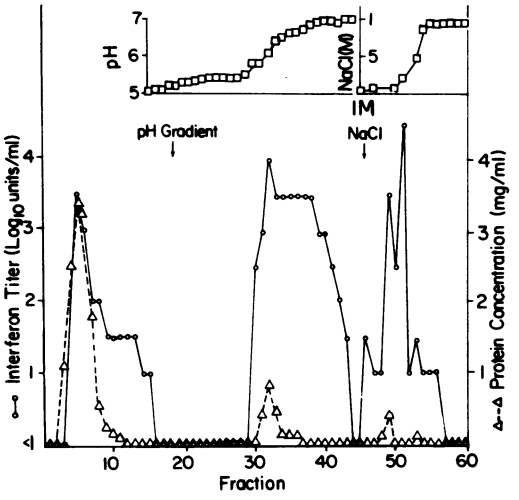

FIG. 3. *Elution profile of mouse immune interferon from BSA-Affi-Gel 10 hydrophobic affinity chromatography. The column was loaded with 85,000 U of interferon in 8.5 ml, washed with 0.05 M sodium acetate, pH 5.0, eluted with a pH gradient of 5 to 7, and finally eluted with 1 M NaCl as indicated on the figure.*

interferons from BSA-Affi-Gel 10 columns were different molecules. Interferon (15,000 U in 7.5 ml) was chromatographed as shown in Fig. 3. Fractions suspected of containing interferon after the pH shift and salt elution steps were independently pooled immediately after collection, dialyzed overnight as described above, and rechromatographed on new BSA-Affi-Gel 10 columns. These pools, designated A and B, were made before their interferon titers were known,

because purified immune interferon is unstable at 4 or −70°C unless exogenous protein, such as BSA, is added. Figures 4A and B represent the elution profiles of rechromatographed interferons. The pH-eluted interferon, pool A, was eluted only by the pH shift during rechromatography (Fig. 4A). Pool B, which had not been eluted by pH previously, was again not eluted by pH shift during rechromatography (Fig. 4B) but was eluted by salt. The data suggest that interferon eluted by pH and salt on this column are distinct molecular entities.

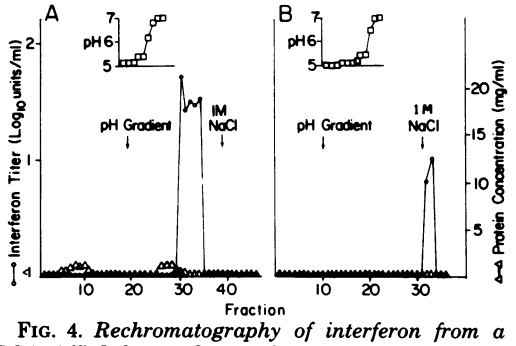

FIG. 4. *Rechromatography of interferon from a BSA-Affi-Gel 10 column, chromatographed as described in the legend to Fig. 3. Fractions of interferon eluted by the pH shift (A) and salt elution (B) steps were pooled, dialyzed against 0.05 M sodium acetate, and rechromatographed separately on new BSA-Affi-Gel 10 columns.*

Stepwise elution from BSA-Affi-Gel 10 columns. We next modified our elution procedure on BSA-Affi-Gel 10 columns in an attempt to increase the purification of interferon. Interferon was applied to the column as before, but eluted sequentially with five buffers. Buffers used for elution were prepared by adding 0.02 M sodium phosphate (pH 7.0) to 0.05 M sodium acetate (pH 5.0) until desired pH values were obtained. Buffers used were: pH 5.2 (buffer A); pH 5.4 (buffer 13); pH 5.4, with 0.08 M NaCl (buffer C); pH 6.0, with 0.08 M NaCl (buffer D); and pH 7.0, with 1 M NaCl (buffer E). There was no sodium acetate in buffer E. The elution profile of this column is shown in Fig. 5. Elution buffers A, B, and D eluted no interferon, although there was some protein eluted. Buffers C and E, both of which contained NaCl, resulted in the elution of interferon. Fraction 60, which was four fractions after buffer C was added, had a specific activity of 24,300 U/mg of protein. It was purified 35-fold. Fraction 73, which was two fractions after buffer E was added, contained 20,000 U of interferon per ml and had a specific activity of 238,000 U/mg of protein. This latter fraction had been purified 325-fold compared with the crude interferon.

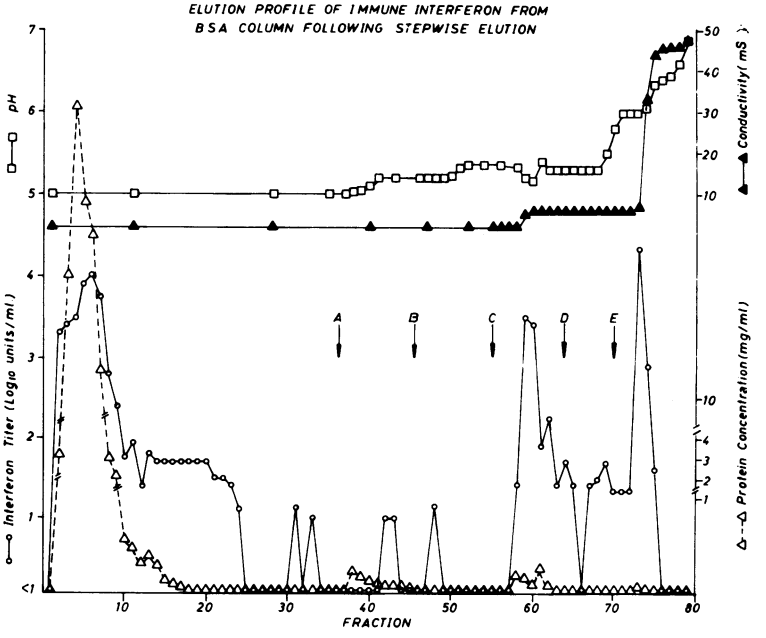

FIG. 5. *Elution profile of mouse immune interferon from BSA-Affi-Gel 10 affinity chromatography. Interferon was added to this column as described in the legend to Fig. 3, but elution was carried out in five steps with the following solutions: (A) 0.05 M sodium acetate (pH 5.0) plus 0.02 M sodium phosphate (pH 7.0) mixed to a pH of 5.2; (B) acetate-phosphate, pH 5.4; (C) 0.08 M NaCl in acetate-phosphate, pH 5.4; (D) 0.08 M NaCl in acetate-phosphate, pH 6.0; (E) 1 M NaCl in 0.02 M sodium phosphate, pH 7.0.*

DISCUSSION

A major problem in the purification of immune interferon is the lack of an in vitro system for large-scale production. We have developed a system for large-scale production of mouse immune interferon. The system involves roller bottle cultures of mouse spleen cells, SEA as the inducer, a cell concentration of 3×10^6 to 5×10^6 cells per ml, and the reducing agent 2-mercaptoethanol. With 3-day incubations, interferon yields varied from $10^{2.7}$ to 10^4 U/ml. The spleen from a 20-g C57Bl/6 mouse yields approximately 1.5×10^8 dissociated cells. Assuming an interferon titer of 10^3 U/ml, it would require approximately 200 mice, then, to produce 10^7 U of immune interferon. This system is convenient for production of immune interferon for purification on a large scale. We have preliminary data on the relative ability of SEA, concanavalin A, and phytohemagglutinin P to induce immune interferon production in human peripheral lymphocyte cultures (22). SEA was the most suitable inducer for the mouse system, as described above, and thus human interferon production should also be possible on a large scale.

The heterogeneity of immune interferon is strongly suggested by the use of three types of chromatography. Ultrogel AcA 34 chromatography resulted in the separation of interferon into two distinct species, one which corresponded to an apparent molecular weight of about 40,000 and another which had a molecular weight of 70,000 to 90,000. Other investigators have reported similar results with Sephadex gel chromatography of antigen-induced interferon (27, 32). More than 90% of the eluted interferon activity was in the molecular weight range of 40,000. Three peaks of interferon activity were found in the elution profile from hydroxylapatite column chromatography. A minor peak was obtained in the breakthrough fraction. Approximately 20% of the recovered interferon was eluted with salt, whereas 75% was eluted with glycerin. Three interferon species were also observed in the elution profile from BSA-Affi-Gel 10 hydrophobic affinity chromatography. Approximately 7% of the recovered interferon was in the breakthrough fraction, whereas 45 and 48% were eluted by pH shift and salt, respectively. Rechromatography of the pH- and salt-eluted species did not alter their elution patterns. Stepwise elution of interferon from the BSA-Affi-Gel 10 columns with buffers of varying pH and salt concentration also resulted in three major species of interferon and additional peaks of minor antiviral activity. Thus, the heterogeneity observed by hydrophobic affinity chromatography apparently represents different species of immune interferon. Heterogeneity was also shown recently by others with mouse immune interferon by using hydrophobic columns (J. Wietzerbin, M. Lucero, S. Stephanos, E. Falcoff, J. O'Malley, E. Sukowski, and W. Carter, personal communication).

Although more than 90% of the immune interferon in the supernatants was precipitated by 80% saturated ammonium sulfate, the minor component precipitated by 55% saturation is being tested to determine its relationship to the 80% precipitate. It is possible that the salt precipitation profile could represent heterogeneity of the immune interferon.

Minor SEA activity was eluted from hydroxylapatite by salt along with one of the interferon species, and also from BSA-Affi-Gel 10 by salt. Immunoadsorption of SEA from the preparations before chromatography, then, provides a convenient method for separation of the inducer from the immune interferon.

The several types of chromatography used in this study resulted in immune interferon fractions of varying degrees of purification and specific activities. On the basis of both recovery and specific activity of recovered interferon, BSA-Affi-Gel 10 hydrophobic affinity chromatography appears to be the most suitable for purification. Selected fractions from the pH shift and salt-eluted column and from the stepwise elution column possessed specific activities of 1×10^5 and 2.4×10^5 U/mg of protein, corresponding to 390- and 325-fold purification, respectively. This is the highest specific activity for immune interferon that has been reported to date. Obviously, the higher the specific activity of the crude interferon, the greater the specific activity of the eluted fractions. Preliminary data on purification using different types of columns sequentially resulted in significant loss of activity and thus made it difficult to determine specific activities of the interferon. The large-scale production system has made it feasible to initiate immunization of rabbits with partially purified immune interferon at doses that correspond to the amounts of virus-type interferon that have been used for antibody production (24, 25).

Recovery of interferon from both BSA-Affi-Gel 10 and hydroxylapatite chromatography was significantly greater than 100%. We feel that this enhanced recovery was not merely the result of experimental error, but was due to the removal of an interferon inhibitor present in the interferon preparation (W. R. Fleischmann, Jr., J. Georgiades, H. M. Johnson, F. Dianzani, and S. Baron, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, S209, p. 247). This inhibitor appears to be made by spleen cells at the same time as immune interferon and may be a feedback control mechanism to limit the biological effects of this interferon.

We have preliminary data which suggest that the culture conditions used for large-scale production of immune interferon are also suitable for large-scale production of lymphotoxin and macrophage migration inhibitory factor (data not shown). Furthermore, the methods used here for purification of immune interferon appear to separate the three types of lymphokine activities, suggesting that they are separate entities. Further studies, however, are required to substantiate these initial observations.

ACKNOWLEDGMENTS

This study was supported by American Cancer Society grant IM-148 and a James W. McLaughlin predoctoral fellowship for research in infectious disease and immunity, awarded to L.C.O.

LITERATURE CITED

1. **Berg, K., C. A. Ogburn, K. Paucker, K. E. Mogensen, and K. Cantell.** 1975. Affinity chromatography of human leukocyte and diploid cell interferons on Sepharose-bound antibodies. J. Immunol. **114**:640–644.
2. **Bergdoll, M. S.** 1970. Enterotoxins, p. 265–326. *In* T. C. Montie, S. Kadio, and S. J. Aijl (ed.), Microbial toxins. Academic Press Inc., New York.
3. **Böhlen, P., S. Stein, W. Dairman, and S. Udenfriend.** 1973. Fluorometric assay of proteins in the nanogram range. Arch. Biochem. Biophys. **155**:213–220.
4. **Brigdon, P. J., C. B. Anfinsen, L. Corley, S. Bose, K. C. Zoon, U. T. Ruegg, and C. E. Buckler.** 1977. Human lymphoblastoid interferon: large scale production and partial purification. J. Biol. Chem. **252**: 6585–5687.
5. **Campbell, J. B., T. Grunberger, M. A. Kochman, and S. L. White.** 1975. A microplaque reduction assay for human and mouse interferon. Can. J. Microbiol. **21**: 1247–1253.
6. **Davey, M. W., E. Sulkowski, and W. A. Carter.** 1976. Purification and characterization of mouse interferon with novel affinity sorbents. J. Virol. **17**:439–445.
7. **DeMaeyer-Guignard, J., M. G. Tovey, I. Gresser, and E. DeMaeyer.** 1978. Purification of mouse interferon by sequential affinity chromatography on poly(U)- and antibody-agarose columns. Nature (London) **271**: 622–625.
8. **Edy, V. G., A. Billiau, and P. DeSomer.** 1977. Purification of human fibroblast interferon by zinc chelate affinity chromatography. J. Biol. Chem. **252**:5934–5935.
9. **Field, A. K., A. A. Tytell, G. P. Lampson, and M. R. Hilleman.** 1967. Inducers of interferon and host resistance. II. Multistranded synthetic polynucleotide complexes. Proc. Natl. Acad. Sci. U.S.A. **58**:1004–1010.
10. **Forsgren, A., and J. Sjöquist.** 1966. Protein A from *Staphylococcus aureus*. I. Pseudo-immune reaction with human-globulin. J. Immunol. **97**:822–827.
11. **Green, J. A., S. R. Cooperband, and S. Kibrick.** 1969. Immune specific induction of interferon production in cultures of human blood lymphocytes. Science **164**: 1415–1417.
12. **Havell, E. A., B. Berman, C. A. Ogburn, K. Berg, K. Paucker, and J. Vilcek.** 1975. Two antigenically distinct species of human interferon. Proc. Natl. Acad. Sci. U.S.A. **72**:2185–2187.
13. **Isaacs, A.** 1963. Interferon. Adv. Virus Res. **10**:1–38.
14. **Jahrling, P. B., and J. L. Beall.** 1977. Chromatographic separations of alphavirus strains by hydroxylapatite. J. Clin. Microbiol. **6**:238–243.
15. **Jankowski, W. J., M. W. Davey, J. A. O'Malley, E. Sulkowski, and W. A. Carter.** 1975. Molecular structure of human fibroblast and leukocyte interferons: profile by lectin and hydrophobic chromatography. J. Virol. **16**:1124–1130.
16. **Johnson, H. M.** 1978. Differentiation of the immunosuppressive and antiviral effects of interferon. Cell. Immunol. **36**:220–230.
17. **Johnson, H. M., and S. Baron.** 1976. The nature of the suppressive effect of interferon and interferon inducers on the *in vitro* immune response. Cell. Immunol. **25**: 106–115.
18. **Johnson, H. M., G. J. Stanton, and S. Baron.** 1977. Relative abilities of mitogens to stimulate production of interferon by lymphoid cells and to induce suppression of the *in vitro* immune response. Proc. Soc. Exp. Biol. Med. **154**:138–141.
19. **Kawakita, M., B. Cabrer, H. Taira, M. Rebello, E. Slattery, H. Weideli, and P. Lengyel.** 1978. Purification of interferon from mouse Ehrlich ascites tumor cells. J. Biol. Chem. **253**:598–602.
20. **Knight, E.** 1975. Heterogeneity of purified mouse interferons. J. Biol. Chem. **250**:4139–4144.
21. **Knight, E.** 1976. Interferon: purification and initial characterization from human diploid cells. Proc. Natl. Acad. Sci. U.S.A. **73**:520–523.
22. **Langford, M. L., G. J. Stanton, and H. M. Johnson.** 1978. Biological effects of staphylococcal enterotoxin A on human peripheral lymphocytes. Infect. Immun. **22**: 62–68.
23. **Maehara, N., M. Ho, and J. A. Armstrong.** 1977. Differences in mouse interferons according to cell source and mode of induction. Infect. Immun. **17**:572–579.
24. **Mogensen, K. E., L. Pyhälä, and K. Cantell.** Raising antibodies to human leukocyte interferon. Acta Pathol. Microbiol. Scand. **83**:443–450.
25. **Ogburn, C. A., K. Berg, and K. Paucker.** 1973. Purification of mouse interferon by affinity chromatography on anti-interferon globulin Sepharose. J. Immunol. **111**: 1206–1212.
26. **Salvin, S. B., J. S. Youngner, and W. H. Lederer.** 1973. Migration inhibitory factor and interferon in the circulation of mice with delayed hypersensitivity. Infect. Immun. **7**:68–75.
27. **Sonnenfeld, G., A. D. Mandel, and T. C. Merigan.** 1977. The immunosuppressive effect of type II mouse interferon preparations on antibody production. Cell. Immunol. **34**:193–206.
28. **Stobo, J., I. Green, L. Jackson, and S. Baron.** 1974. Identification of a subpopulation of mouse lymphoid cells required for interferon production after stimulation with mitogens. J. Immunol. **112**:1589–1593.
29. **Sulkowski, E., M. W. Davey, and W. A. Carter.** 1976. Interaction of human interferons with immobilized hydrophobic amino acids and dipeptides. J. Biol. Chem. **251**:5381–5385.
30. **Törma, E. T., and K. Paucker.** 1976. Purification and characterization of human leukocyte interferon components. J. Biol. Chem. **251**:4810–4816.
31. **Wheelock, E. F.** 1965. Interferon-like virus-inhibitor induced in human leukocytes by phytohemagglutinin. Science **149**:310–311.
32. **Youngner, J. S., and S. B. Salvin.** 1973. Production and properties of migration inhibitory factor and interferon in the circulation of mice with delayed hypersensitivity. J. Immunol. **111**:1914–1922.