Human Leukocyte Interferon Produced by Use of an Antibody-Free Human Serum Fraction

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An antibody-free human serum fraction has been prepared by ammonium sulfate precipitation. This fraction can be substituted for whole serum in the production of optimal yields of interferon by induced human leukocytes.

Human leukocyte suspensions produce interferon after induction with a suitable inducer (4). Optimal yields of interferon are obtained only in the presence of serum (6); lower yields are produced in the absence of serum. Although whole serum can be present in interferon for evaluation of interferon against viral infections, serum antibodies may complicate interpretation of the results (1). Milk and nonhuman sera can be substituted for human serum in interferon production (3, 5–8), but foreign proteins in these materials are unacceptable for use in man. This report describes the preparation of human leukocyte interferon by use of an antibody-free human serum fraction. (Presented in part at the Annual Meeting of the American Society for Microbiology, 23–24 April 1972, Philadelphia, Pa.)

MATERIALS AND METHODS

Viruses. Parainfluenza I/Sendai was grown in 10-day embryonated chicken eggs and polio I/Brundhilde was grown in HeLa cell monolayers.

Leukocytes. Buffy coats were used within 24 h of collection at the New York Blood Center. Red blood cells were removed by treatment with 0.83% ammonium chloride (6).

Interferon assay. Interferon titers were determined by plaque reduction of poliovirus in A-aminon cell monolayers. The cells were grown in Eagle’s minimal essential medium with 10% fetal calf serum. Amounts of 8 ml of cell suspension containing 1.25 × 10^9 cells/ml were dispensed into 60-mm plastic petri plates. The plates were incubated at 37 C in an atmosphere of 5% CO_2 and air for 4 days. The human interferon international standard 69/19, assigned 5,000 units, titrated 1,700 units when assayed in this system.

Interferon production. Leukocyte interferon was produced by methods already published (4, 6). Leukocytes were suspended in Eagle’s basal medium, buffered with 0.05 M tricine at pH 7.4 and supplemented with 5% heat-inactivated serum or serum substitute. The cell suspension, containing 10^9 cells/ml, was primed with 50 to 100 interferon units per ml and incubated for 2 h at 37 C. Parainfluenza I, 300 hemagglutinating units/ml, was added, and the incubation was continued for 10 to 18 h. The suspension was centrifuged at 5,000 × g for 10 min, and the supernatant fluid was dialyzed at 4 C for 48 h against 0.05 M KCl at pH 2 to inactivate residual virus. The dialysate was centrifuged at 10,000 × g for 10 min, and the pH was adjusted to 7.2 by dialysis against 0.01 M sodium phosphate buffer.

Gel filtration chromatography. Sephadex G-200 was packed at 25 C in a column 2 cm in diameter to a bed height of 45 cm. The column was equilibrated with 0.01 M sodium phosphate buffer at pH 7.2 at a flow rate of 20 ml/h. Eluent fractions of 3 ml were collected at the same flow rate. Protein was monitored by ultraviolet absorption at 280 nm.

Polyacrylamide gel electrophoresis. An E-C 470 apparatus (E-C Apparatus Corp., Philadelphia, Pa.) was used for electrophoretic analysis. Samples of serum or interferon, mixed with sucrose to a final concentration of 10%, were applied to 5% gels in tris(hydroxymethyl)aminomethane-borate buffer at pH 8.2. Electrophoresis was run at 300 V for 2 h. The gels were stained with 0.1% Amido Black in 20% methanol and 7% acetic acid. The methanol-acetic acid mixture was used for destaining the gels.

Detection of antibodies. Low-level ImmunoPlate tests (Hyland, Division Travensol Laboratories, Inc., Costa Mesa, Calif.) were used. The minimal amounts of immunoglobulins A, G, and M (IgA, IgG, and IgM) that could be detected by this method were 0.042, 0.045, and 0.035 mg/ml, respectively.

Preparation of an antibody-free serum fraction. Human serum was inactivated by incubation at 56 C for 30 min, and solid ammonium sulfate was added to 50% saturation. After 2 h at 4 C, this was centrifuged for 10 min at 10,000 × g. The antibody-free supernatant fluid was dialyzed overnight against pH 7.2, 0.01 M phosphate buffer. The precipitate was reconstituted in pH 7.2, 0.01 M phosphate buffer and dialyzed overnight against the same buffer.

RESULTS

Substitution of ammonium sulfate fractions for whole serum. The data in Table 1 show that both the supernatant fluid and the
precipitate supported interferon production at levels attained with whole serum. The precipitate from the 25% ammonium sulfate saturation did not support interferon production; high interferon yields, comparable to controls, were obtained when the supernatant fluid from this fractionation was used.

**Reduction of immunoglobulins after 50% ammonium sulfate fractionation.** Immunodiffusion studies showed that no antibodies were present in either the ammonium sulfate supernatant fluid or the interferon samples prepared with this fraction. Based on these studies, interferon with 5% serum contains approximately 0.33 mg of IgG/ml. After (NH$_4$)$_2$SO$_4$ fractionation, the interferon contained less than 0.045 mg of IgG/ml, the low limit of sensitivity for the test. Similarly, IgA and IgM were not detectable at sensitivity limits of 0.042 and 0.035 mg/ml, respectively.

**Characterization of the ammonium sulfate supernatant fluid.** Whole serum and the ammonium sulfate fraction were chromatographed on Sephadex G-200 gel. Their elution profiles are shown in Fig. 1. The small front peak of the ammonium sulfate sample did not contain protein, as shown by the ratio of absorption at 260 and 280 nm, but its identity was not established because of insufficient material; acrylamide gel electrophoresis showed that the major component of the ammonium sulfate-treated sample was albumin. The early fractions (16–21) and the late fractions (22–30) from the ammonium sulfate gel chromatography gave identical electrophoretic bands, showing that the 50% ammonium sulfate supernatant fluid did not contain any high-molecular-weight material associated with globulins. That the gel chromatography products of whole serum were not homogeneous was shown by the lack of symmetry of the corresponding fractions.

When albumin was substituted for serum in the leukocyte reaction, the interferon yield was 3,000 units as compared to the original 18,000.

**Table 1. Comparison of interferon production yields in presence of various fractions of human serum**

| Serum fraction                  | Interferon titer/ml |
|--------------------------------|---------------------|
| Human serum 50% (NH$_4$)$_2$SO$_4$ supernatant fluid | 20,000              |
| Human serum 25% (NH$_4$)$_2$SO$_4$ supernatant fluid | 15,000              |
| Human serum 50% (NH$_4$)$_2$SO$_4$ precipitate | 15,000              |
| Human serum 25% (NH$_4$)$_2$SO$_4$ precipitate | 1,000               |
| Whole serum                     | 18,000              |

**DISCUSSION**

The ammonium sulfate supernatant fluid described in this paper is a serum substitute which is free from serum antibodies and which allows for optimal production of human leukocyte interferon. Interferon prepared with this serum fraction rather than whole serum was easier to concentrate and to reconstitute after lyophilization. Although albumin is the fraction’s major component, it alone does not support production of interferon. It is possible that additional minor serum components, alone or with albumin, are needed for optimal production in this system. It is also possible that methods other than ammonium sulfate precipitation used to prepare albumin have a denaturing effect on the albumin.

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