The Production of Human Leukocyte Interferon in a Serum-Free Medium (36954)

MOSHE Y. GOORE, JUDITH H. DICKSON, SAUL LIPKIN, AND CONSTANTINO J. DICUOLLO
(Introduced by R. F. Haff)
Research and Development Division, Smith Kline and French Laboratories, Philadelphia, Pennsylvania 19101

It has been reported that induced human leukocyte suspensions produce little interferon in a serum-free medium, while substantial quantities are produced in the presence of serum (1, 2). Interferon preparations containing serum, unless purified, may not be suitable for clinical evaluation against infectious agents because of the presence of specific antibodies in the serum (3). Furthermore, chemical and physical characterization of interferon and studies on the mechanism of its action will require highly purified preparations. Although many purification procedures of interferon are available, significant losses during the various purification steps are unavoidable (4–6). Thus interferon prepared without serum, if available, could provide material with higher initial purity. These preparations could be used in clinical trials without concern for antibody and they would be much easier to purify. This report describes the preparation of human leukocyte interferon in a serum-free medium, in yields comparable to those obtained in the presence of serum.

Materials and Methods. Viruses. Parainfluenza I/Sendai was grown in 10-day-old embryonated hen eggs and polio I/Brunhilde was propagated in HeLa cell monolayers in roller bottles.

Leukocytes. Buffy coats, obtained from the New York Blood Center, were utilized within 24 hr of collection time. Red blood cells were removed by treatment with 0.83% ammonium chloride (7).

Interferon assay. Interferon titers were determined by plaque reduction of poliovirus in U-amnion cell monolayers. The cells were grown in Earle's minimal essential medium, supplemented with 10% fetal-calf serum. Eight milliliters of the cell suspension, containing 1.25×10^5 cells/ml, were dispensed into 60 mm plastic Petri plates. The plates were incubated at 37° in an atmosphere of 5% CO_2 and air for 4 days before use. The human interferon international standard 69/19, assigned 5000 units, titrated 1700 units when assayed by this method. All titers are expressed as units per ml.

Interferon production. Leukocyte interferon was produced essentially by methods already published (2, 8). Leukocytes were suspended in Earle's basal medium (BME), buffered with tricine at pH 7.4 to a final concentration of 0.05 *M*, and supplemented with 5% heat-inactivated serum. The leukocyte suspension, at 10^7 cells/ml, was primed with 100 interferon units per ml (9) and incubated for 2 hr at 37° with gentle stirring. After priming, 300 hemagglutinating units/ml of parainfluenza I were added to induce the cells, and the incubation was continued for 10 to 18 hr. The suspension was centrifuged at 4° at 5000*g* for 10 min and the supernatant was dialyzed at 4° for 48 hr against a solution of 0.05 *M* KCl at pH 2 to inactivate residual virus. It 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 for 10 to 12 hr.

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

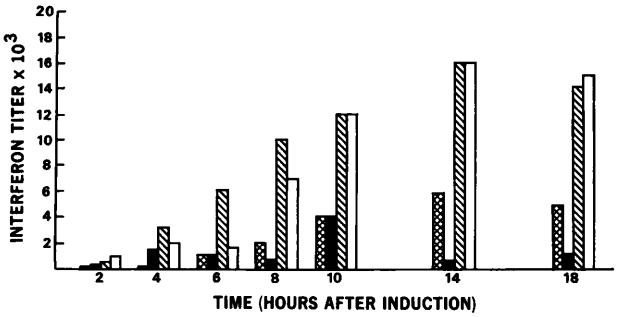

FIG. 1. Rate of interferon production by leukocytes in the presence and absence of serum. Hatched bars, interferon production by leukocytes in the presence of serum by unprimed leukocytes; solid bars, interferon production in serum-free medium by leukocytes primed in the absence of serum; striped bars, interferon production in serum-free medium by leukocytes primed in the presence of serum; blank bars, interferon production in the presence of serum by leukocytes primed in the absence of serum. Interferon titers are expressed as units per ml.

staining the gels.

Detection of globulins. Low level Immuno-Plate tests (Hyland, Division Travenol Laboratories Inc., Cal.) were used. A minimum of 0.042 mg/ml IgA, 0.045 mg/ml IgG and 0.035 mg/ml IgM could be detected by this method.

Protein determination. Protein was determined by the colorimetric method of Lowry (10).

Results. Preparation of interferon in a serum-free medium. Leukocytes were suspended in BME buffered with tricine and supplemented with serum as described in Methods. The cell suspension, at a concentration of 10^7 cells/ml, was primed and incubated for 2 hr at 37° with gentle stirring. Following priming, the medium containing the serum was removed by centrifugation at room temperature at 200*g* for 20 min. The cells were resuspended in prewarmed BME, buffered with tricine, containing no serum. Induction and harvest were performed as described in Methods. As shown in Fig. 1, the kinetics of interferon production in the presence and absence of serum were essentially the same when the cells were primed in the presence of serum. However when serum was absent during the priming period, low levels of interferon were produced. For comparison, the rate of interferon production by unprimed leukocytes in the presence of serum and by primed leukocytes in the absence of serum are also shown in Fig. 1. Under both conditions the levels of interferon produced were

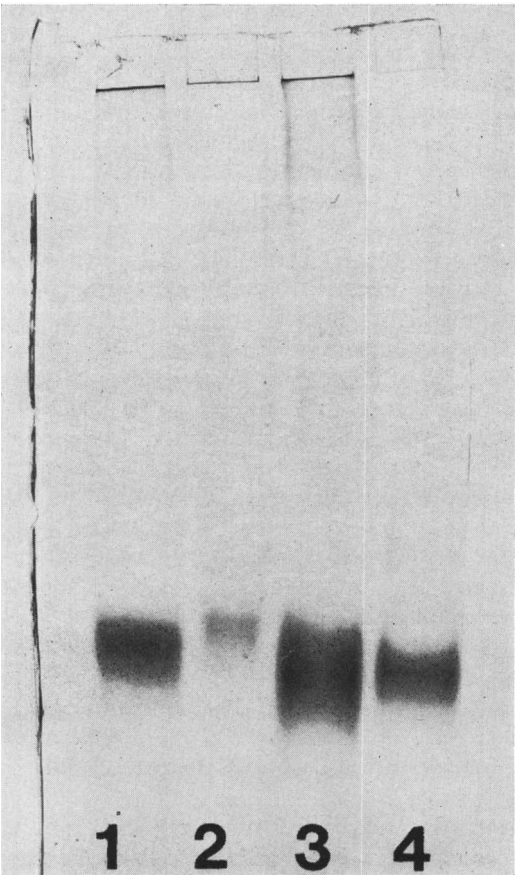

FIG. 2. Acrylamide gel electrophoresis of leukocyte interferon prepared with and without serum. (1) Human serum, (2) serum-free interferon, 100-fold concentrated, (3) serum interferon, (4) crystalline human albumin.

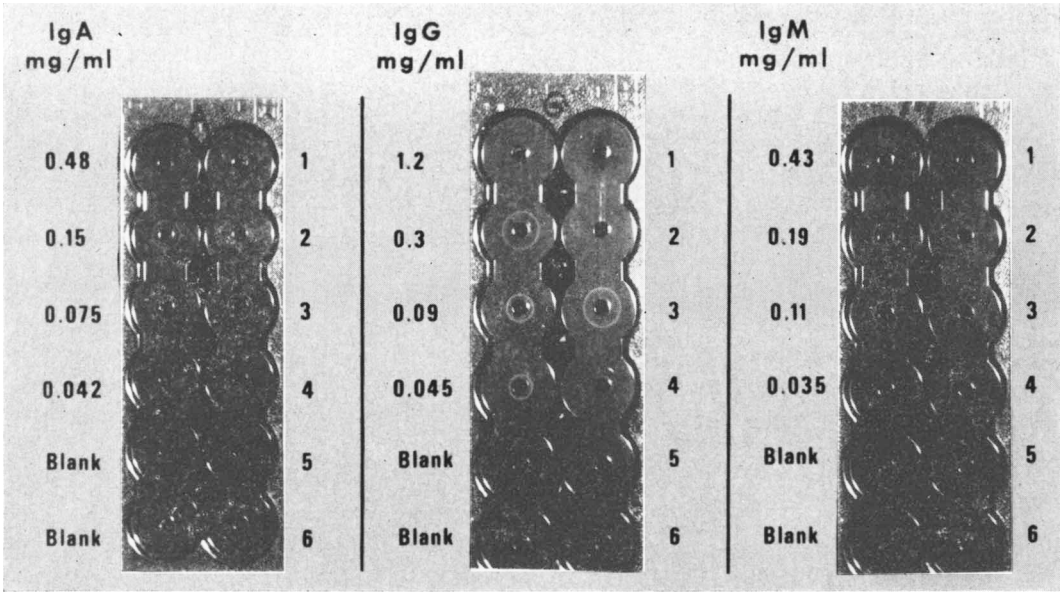

FIG. 3. Globulin content of interferon samples prepared with and without serum. (1) Human serum, (2) serum-free interferon, (3) interferon prepared with serum.

much lower than those produced by leukocytes primed in the presence of serum, whether the induction and the production processes were carried on with or without serum. Although more studies are needed to delineate the precise role of serum in the production of interferon by leukocytes, these data indicate that its role is mainly restricted to the priming event.

The protein levels of interferon prepared with and without serum were found to be 2.9 mg/ml and 0.2 mg/ml, respectively. Thus the serum-free preparations contain at least 15-fold less protein than interferon prepared with serum.

Characterization of proteins contained in serum-free interferon. As stated above serum-free interferon preparations contained approximately 0.2 mg/ml protein. Since a protein-free medium was used in the preparation of this interferon, the protein detected in these samples could have been released to the medium from lysed cells or cell contamination. The nature of this protein was established by acrylamide gel electrophoresis. The electrophoretic behavior of this protein, shown in Fig. 2, indicate that it consists mainly of albumin.

Absence of gammaglobulin from the serum-free interferon preparation. Although the nature of the protein present in the serum-free interferon preparations could be established by acrylamide gel electrophoresis, the method is not specific for antibodies. To determine the globulin content of the serum-free interferon preparations, immunodiffusion was used. In these experiments, purified IgA, IgG and IgM were used as standards. The results shown in Fig. 3 indicate that no globulins could be detected in these preparations. As expected, interferon prepared with serum contained all of the three major components of globulins.

Discussion. Hadharzy *et al.* (1) reported that human leukocytes require serum to produce optimal quantities of interferon. Very low yields of interferon are obtained when serum is not included in the leukocytes suspension. The present studies partially confirm this observation. They demonstrate however that interferon is produced in high yields in the absence of serum, when the cells are previously primed in the presence of serum. Although the serum requirement is still poorly understood, these studies indicate that the presence of serum is essential during the priming process. Thus a better understanding of the priming mechanism should help explain the serum requirement for optimal

production of interferon by induced leukocytes.

In a previous study we have demonstrated that high yields of leukocyte interferon could be obtained by supplementing leukocyte suspensions with an antibody-free human serum fraction instead of whole serum (11). Such preparations, although suitable for clinical evaluation of interferon, still contained high levels of extraneous protein. The serum-free interferon preparation described in this report, in addition to being free of antibody, offers the added advantage of having an initial lower protein concentration. This is an important factor when concentration and purification of interferon is contemplated.

Summary. Human leukocyte interferon was prepared in a serum-free medium. The yields were comparable to those obtained with serum, provided that the cells were first primed with low concentration of interferon in the presence of serum.

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Received Sept. 6, 1972. P.S.E.B.M., 1973, Vol. 142.