Characterization of the Size and Charge Heterogeneities of Human Leukocyte Interferon Populations

Brief Report

By

L. S. LIN, MARZENNA WIRANOWSKA-STEWART, T. CHUDZIO, and W. E. STEWART II
Interferon Laboratories, Memorial Sloan-Kettering Cancer Center New York, New York, U.S.A.

With 3 Figures

Accepted September 8, 1977

Summary

Human leukocyte interferons are separable into two size components by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and are separable into two charge-components by DEAE-BioGel A chromatography. However, each of the charge-components resolved by ion-exchange chromatography contained both size-components, when analysed by SDS-PAGE. Thus, there are more than two distinct molecular populations of human leukocyte interferons.

Human leukocyte interferon (HuLeIF) preparations contain two distinct molecular populations of interferons which can be separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), one population with a peak of activity at 21,000 daltons and one with a peak of activity at 15,000 daltons (2). These components can also be distinguished by their relative abilities to induce antiviral activity in heterologous (rabbit) cells and their stabilities in ionic detergents under reducing conditions (2). Isolation of sufficient quantities of each of these size-populations of HuLeIFs to analyse the nature of their heterogeneities has been laborious because of limitations in quantities applicable to and recoverable by analytical SDS-PAGE. Recently, however, it was reported that two populations of HuLeIF could also be separated from HuLeIF preparations by ion-exchange chromatography (1). It was suggested that the two charge-heterogeneous interferons were the same components reflected in the two size populations, and it was claimed that this procedure afforded the first clear-cut separation of the two components of HuLeIF without resorting to denaturing conditions (1). As such a separation scheme would also circumvent the quantitative...
restrictions imposed on separating the two HuLeIF size-components by SDS-PAGE, we undertook to determine the validity of this interpretation.

HuLeIF, prepared from human leukocytes induced with Sendai virus, was obtained from Dr. K. Cantell (Helsinki, Finland) and contained approximately 6 × 10^6 units/ml and 10^6 units/mg of protein. This material was subjected to electrophoresis in SDS-polyacrylamide gels as previously described (2) and was found to be size-heterogeneous, with peaks of activity at 21,000 daltons and 15,000 daltons (Fig. 1). Each component isolated by SDS-PAGE was found to re-electrophorese to the same position to which it had previously migrated (Fig. 1, A—E); thus no smaller molecular forms were generated from the larger ones by boiling in SDS. In fact, the re-electrophoresis of each of the various size-components to its original position suggests that the size-heterogeneity is more than two distinct size classes, but may rather be a continuous gradient of molecules with different sizes.

Fig. 1. Electrophoresis of human leukocyte interferon in SDS-polyacrylamide gels. Human leukocyte interferon was diluted to contain about 6 × 10^6 units/ml in 0.01 mM phosphate buffer, pH 7.1, and was constituted to contain 1 per cent SDS, 5 M urea and was heated at 100°C for 1 minute. Aliquot of 0.1 ml was applied to 20 cm cylindrical SDS-polyacrylamide gel. Following electrophoresis, fractions (0.44 cm) were eluted into Eagle's minimal essential medium (MEM) containing 10 per cent fetal calf serum and assayed for interferon on human fibroblast cultures by inhibition of cytopathic effect of vesicular stomatitis virus. Units are approximately equivalent to NIH human leukocyte interferon reference units when titrated against reference reagent GO-23-901-527. Approximately 113 per cent activity was recovered from the gel electrophoresed with the total HuLeIF preparation. Fractions A, B, C, D and E (designated by black boxes) were again constituted to contain 1 per cent SDS, 5 M urea, were heated, and 0.1 ml aliquots were electrophoresed on parallel SDS-polyacrylamide gels (A, B, C, D, E) fractionated and assayed as before.