Assessment of the Interferon-Like Activity in Preparations of Leukocyte Pyrogen

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SUMMARY

Preparations of crude leukocytes from rabbit peritoneal exudates contain interferon-like activity which may be separated from pyrogen activity both by Sephadex chromatography and by purification of the pyrogen. This indicates that, despite some similarities, leukocyte pyrogen is not an interferon.

Interferons are substances produced by animal cells which confer resistance to virus infection upon normally susceptible cells. They have many characteristics in common with leukocyte pyrogens, substances produced by granulocytes which induce brief, monophasic fevers. Both pyrogenic responses and interferon can be induced in animals by administration of endotoxins and viruses (Atkins, 1960; Baron & Buckler, 1963; Ho, 1964; Stinebring & Youngner, 1964; Rafter, 1969; Grossberg, 1972). In addition, production of both interferon and pyrogen declines progressively on repeated stimulation by either endotoxins or viruses, a phenomenon called tolerance (Beeson, 1947; Bennett et al. 1949; Ho & Kono, 1965; Ho et al. 1965; Siegert et al. 1967). Both pyrogen and interferon are proteins which display some species specificity (Bornstein & Woods, 1969; Grossberg, 1972; Lockhart, 1974).

Several groups of investigators have compared pyrogen and interferon production in the same animals. Siegert et al. (1967) showed that Newcastle disease virus (NDV) injected into rabbits induced serum levels of pyrogen and interferon simultaneously and that the development of tolerance occurred with similar time courses for both. Shu & Tan (1970) studied the appearance of NDV-stimulated interferon and pyrogen in individual organs of stimulated rabbits. They found that time courses of appearance were similar in all organs tested except the spleen. An attempt was made by Kohlhage et al. (1968) to separate interferon in rabbit serum from leukocyte pyrogen by means of ethanol precipitation. This was not completely accomplished, although pyrogen and interferon were shown to have somewhat different sensitivities to mercaptoethanol and low pH treatment.

The isolation of rabbit leukocyte pyrogen in pure form by Murphy et al. (1974) makes it possible to determine whether purified leukocyte pyrogen has interferon-like activity. In this communication we show that crude preparations of pyrogen produced by endotoxin stimulation of peritoneal exudate leukocytes contain small amounts of interferon-like activity, which can be separated from pyrogen activity by Sephadex chromatography. We also show that purified pyrogen preparations do not have any interferon activity.

Batches of crude leukocyte pyrogen were produced and assayed for pyrogen activity by the method of Murphy et al. (1971). Amounts of pyrogen were expressed in terms of the increase in body temperature, measured in °C, produced by 1 ml of sample. Antiviral activity was measured in a plaque reduction assay in primary rabbit kidney cells. A standard interferon produced in our laboratory by NDV stimulation of RK-13 monolayer cultures was included as a control for the sensitivity of the batch of primary rabbit kidney cells used. This had been purified by the method of Carver et al. (1968) and usually gave a titre of 1000 units. This standard interferon had no pyrogenic activity at the maximum concentrations
Fig. 1. Sephadex G-100 fractionation of concentrated pyrogen preparations. •—•, Absorbance at 280 nm; ○—○, interferon units as measured on primary rabbit kidney cells; △—△, rabbit leukocyte pyrogen, °C/ml. This graph indicates that leukocyte pyrogen is separable from interferon activity on the column.

Crude pyrogen samples containing 7.8 to 10.2 °C of activity showed from 1 to 5 units of interferon-like activity as measured in four separate assays. The antiviral activity was trypsin sensitive and there was no direct antiviral effect when crude preparations were mixed with vesicular stomatitis virus (Indiana strain) prior to plaquing.

Because of their low levels of interferon-like activity, crude pyrogen preparations were concentrated 100-fold before being chromatographed on a Sephadex G-100 column. Interferon-like activity, pyrogen content and protein concentrations were determined on effluent fractions. Fig. 1 illustrates the separation attained. Most of the interferon activity eluted in a broad band which preceded the band containing the pyrogen activity. Interferon activity was not found at the pyrogen peak. There was, however, a small amount of interferon activity eluting in two peaks immediately preceding and following the pyrogen peak. Therefore it would seem that the small interferon peak was not an artifact.

Additional batches of crude pyrogen were purified as described by Murphy et al. (1974) and then further purified by chromatography on CM-cellulose using a gradient of sodium acetate buffer, pH 5.0 and on hydroxylapatite using a sodium phosphate buffer, pH 6.5. CM-cellulose purified material, with a specific activity of 1000 °C/mg contained no interferon activity when a sample containing 24 °C of pyrogenic activity was tested. Hydroxylapatite purified pyrogen, with a specific activity of 10000 °C/mg also contained no interferon activity when samples containing 169 °C of pyrogenic activity were tested. Smaller amounts of each of the purified pyrogens were tested in separate trials. These also failed to show interferon activity.

We have shown that although there are some similarities between rabbit leukocyte
pyrogen and rabbit interferon, the two are separable by Sephadex chromatography and, as leukocyte pyrogen is purified, all detectable interferon-like activity disappears, indicating that leukocyte pyrogen is not in fact an interferon.

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