Title
Biochemical studies of high molecular weight complex lymphotoxin and evidence that it is involved in cytotoxic T-cell mediated lysis

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Authors
Devlin, James J
Klostergaard, Jim
Yamamoto, Robert S
et al.

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to 45,000. The purified cell line product behaved similarly to tumor necrosis serum cytotoxin in all comparative studies: pH optimum, heat and chemical inactivation, differential target cell sensitivity, and the ability of metabolic inhibitors and culture conditions to influence target cell sensitivity. Metabolic and electron microscopic studies suggested that target cells needed to internalize the cytotoxin.

Purification and Characterization of Lymphotoxin from Human Lymphoblast Cell Line 1788. B. B. AGGARWAL, B. MOFFAT, AND R. N. HARKINS, Department of Protein Biochemistry, Genentech, Inc., South San Francisco, California 94080.

Human lymphoblast cell line RPMI-1788 was grown in a serum-free medium and the lymphotoxin was isolated from the culture medium and characterized. The ultimate purification scheme will be discussed. The characterization of the material was performed by reverse-phase high-pressure liquid chromatography, SDS and native polyacrylamide gel electrophoresis, gel filtration, and by amino terminal sequence analysis. A complete tryptic digest of the molecule provided two distinct peaks on reverse-phase HPLC. The partial amino terminal sequence of the fragments will be presented.

Biochemical Studies of High Molecular Weight Complex Lymphotoxin and Evidence that it is involved in Cytotoxic T-Cell Mediated Lysis. JAMES J. DEVLIN, JIM KLOSTERGAARD, ROBERT S. YAMAMOTO, AND GALE A. GRANGER. Department of Molecular Biology and Biochemistry, University of California, Irvine, Irvine, California 92717.

Lymphotoxins (LT) are a family of cytolytic glycoproteins produced by mammalian lymphocytes *in vitro*. Complex LT is the highest molecular weight (>200,000 daltons) and one of the most cytolytic classes of LT. We have found that antisera to partially purified complex LT inhibit human cytotoxic T-cell-mediated lysis *in vitro*, thereby implicating complex LT in lymphocyte-mediated lysis. Our results also suggest that complex LT can be associated with an antigen receptor. We have demonstrated that antisera to the $F(ab')_2$ region of human IgG recognize complex LT. Additionally, complex LT from antigen-stimulated lymphocytes can be specifically adsorbed to affinity columns on which the stimulating antigen has been immobilized. By the sequential use of lectin and hydrophobic affinity chromatography, molecular sieving, and isoelectric focusing, we have purified ^{125}I-labeled complex LT from serum-free media at least 20,000-fold. Antiserum we are raising against this highly purified preparation of complex LT will be used to more precisely determine the role of these molecules in cell-mediated cytolysis.

Purification of Leukocyte Inhibitory Factor Derived from a Human Non-T, Non-B Cell Line. D. MESHULAM AND R. E. ROCKLIN, Tufts–New England Medical Center, Boston, Massachusetts.

Leukocyte inhibitory factor (LIF), a lymphokine that inhibits the random and directed migration of polymorphonuclear (PMN) leukocytes, was purified from a human non-T, non-B leukemia cell line (Reh). From 10 liters of serum-free supernatant, 1.3 μg of protein with LIF activity was obtained by the sequential use of affinity chromatography with Con A–Sepharose, hydrophobic chromatography with hexylagarose, and gel filtration chromatography. The specific activity of LIF recovered represented an 80,000-fold purification over that of the initial crude serum-supernatants, and the preparation at that point was estimated to be 80–90% pure. They both assess the purity of the preparation and provide a further purification step; Reh LIF activity recovered by the above procedures was subjected to isoelectric focusing. One major stainable protein band was identified; its isoelectric point was pH 5.4–5.5. Gels run in parallel for recovery of biologic activity revealed only one region (pH 5.4–5.5) with ability to inhibit PMN leukocyte migration. Iodination of Reh LIF resulted in a loss of biologic activity, but isoelectric focusing of this material revealed one major ^{125}I-labeled band (pH 5.1) and several minor bands. The coincidence of biologic LIF activity with one stainable protein band as identified by isoelectric focusing implies that the final product may be homogeneous.

Application of a Standardized LIF Assay to Evaluate Lymphokine Activity of Interferons. ANNE S. HAMBLIN, URSULA SHIPTON, AND *ALAN MORRIS, Department of Immunology, St. Thomas' Hospital, London, and *Department of Biological Sciences, University of Warwick, United Kingdom.

There is increasing evidence that interferons (IFNs) exert immunoregulatory activity by virtue of lymphokine-like activities. In preliminary work we have found that human gene-cloned IFNα_2, purified by affinity chromatography using a monoclonal antibody, and partially purified IFNγ, both have mi-