Title
The PGLC-33H cell line appears to secrete only a-Lymphotoxin

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Big OAF elutes between the molecular weight markers, chymotrypsinogen (25,000) and cytochrome *c* (12,500), and Little OAF elutes from the same columns with [^{3}H]proline (140). Big OAF is dissociated to Little OAF after equilibration with 1 *M* NaCl or 2 *M* urea. Little OAF is reaggregated back to Big OAF when equilibrated in buffers of low ionic strength. Little OAF is extracted into the organic phase in ethyl acetate acidified to pH 3.5 and remains in the aqueous phase at pH 7.5. The dissociation–reaggregation properties of OAF and its behavior in organic solvents are aiding in its chemical purification.

The PGLC-33H Cell Line Appears to Secrete Only α-Lymphotoxin. D. S. FAIR, E. W. B. JEFFES, III, C. F. WARE, AND G. A. GRANGER, University of California, Irvine, California.

We characterized lymphotoxin (LT) components present in supernatants of the human lymphoid cell line PGLC-33H. Because we found a single, stable 80,000–90,000 molecular weight cytotoxin, PGLC supernatants appear to contain α-LT and no detectable β-LT. Fractionation of this material over phosphocellulose, DEAE-cellulose, or in PAGE gels detected one major and at least two minor components. This heterogeneity was similar to α-LT present in the supernatants from mitogen-activated lymphocytes. PGLC-LT activity could be neutralized by antisera directed toward α-LT, obtained from mitogen-activated lymphocytes on mitomycin C-treated L-929 cells. While the PGLC supernatants' growth inhibited both L-929 and HeLa cells, the addition of antisera completely neutralized this effect on L-929 cells, but only partially neutralized growth inhibition on HeLa cells. If PGLC supernatants wre heated to 56°C/1 hr and antisera was added, the growth inhibition on HeLa cells was completely abrogated, indicating a second, unstable regulatory factor. These data indicate that: (1) PGLC cell line secretes a family of α-LT, which resembles wild-type α-LT physically, antigenically, and biologically; (2) although α-LT accounts for the growth inhibition activity detected on L-cells, at least one other component can be demonstrated on HeLa cells; (3) PGLC supernatants contain a less complex mixture of lymphokines.

Source, Function, and Properties of Murine and Human T-Cell-Replacing Factors (TRF). A. SCHIMPL, E. WECKER, L. HÜBNER, TH. HÜNIG, AND G. MÜFLER, University of Würzburg, Federal Republic of Germany.

An antigen nonspecific soluble factor produced by θ $^+$F$_c$-Murine T-lymphocytes, allogeneically or Con A stimulated, reconstitutes both IgM and IgG *in vitro* immune responses to various antigens. TRF acts even on anti-θ and complement-treated spleen cultures from nu/nu mice. Kinetic and autoradiographic studies show that TRF converts B-cells already proliferating upon a previous antigen contact into Ab-producing/secreting cells and seems to represent a third signal distinct from induction and proliferation signals. TRF is a protein (MW ~30,000), does not contain H-2-determined antigens, does not bind to various lectin columns, and is not inactivated by neurominidase. It can be partially purified by gel filtration, hydrophobic chromatography, and PAGE. A functionally similar factor with a similar MW can be obtained from allogeneically or Con A-stimulated human embryonic spleen cells or from human tonsil cells. It reconstitutes the *in vitro* immune response of B-cell-enriched human tonsil cells to heterologous blood cells. Both factors show a species preference.

SESSION VIII: Biological Phenomena and Relevance

Activated Macrophage–Lymphocyte Interaction through Soluble Mediators. M. S. MELTZER, J. J. OPPENHEIM, L. RUCO, AND E. J. LEONARD. National Institutes of Health, Bethesda, Maryland.

Macrophage–lymphocyte interactions occur throughout afferent limb and effector immune reactions to amplify responses to weak immunological stimuli. These interactions are mediated, in part, by soluble products from lymphocytes and macrophages. Activated macrophages are particularly efficient in amplification reactions; these cells show both increased responsiveness to and increased elaboration of soluble mediators. *In vitro* chemotaxis of peritoneal macrophages from mice treated with agents which induce tumoricidal activated macrophages to chemotactic factors present in supernatants of antigen-stimulated lymphocytes was two to five times greater