Lymphotoxin Production in Human Neoplasia[1] (35580)

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(Introduced by B. R. Forsyth)

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The *in vitro* stimulation of lymphocytes by both specific antigens and nonspecific factors such as phytohemagglutinin is accompanied by the release of a soluble toxic substance which has been termed lymphotoxin (1). Lymphotoxin is assayed by *in vitro* target cell destruction and its appearance has been correlated with the capacity of lymphocytes to be activated and undergo the morphologic change referred to as lymphocyte transformation (2). Human lymphotoxin appears to be a protein with a molecular weight of approximately 85,000 (3).

Hodgkin's disease is associated with a specific depression of delayed hypersensitivity. Patients with Hodgkin's disease are unable to react positively to contact sensitization by a new antigen such as dinitrochlorobenzene (4). Patients with Hodgkin's disease also reject skin homografts much more feebly than do normal control patients (5), and patients with Hodgkin's disease respond less than a control population to intradermal skin testing with common skin test antigens (6). Thus all aspects of delayed or lymphocyte hypersensitivity appear to be depressed in Hodgkin's disease. Since *in vitro* lymphocyte transformation has also been observed to be impaired in patients with Hodgkin's disease (7), the purpose of the present investigation was to determine the ability of patients with Hodgkin's disease to form lymphotoxin in response to phytohemagglutinin. Normal noncancer patients and patients with malignancy other than Hodgkin's disease were similarly studied.

Materials and Methods. Human peripheral blood lymphocyte cultures were established following venipuncture by a previously described method (8). Blood samples were obtained from 16 patients with Hodgkin's disease, 26 patients with cancer other than Hodgkin's disease, and 20 control patients without neoplasm hospitalized at the Medical Center Hospital of Vermont. None of the Hodgkin's patients were receiving chemotherapy or radiation therapy at the time of venipuncture and none of the cancer patients were in the terminal phase of their illness. After 5 days in culture, sterile cell-free supernatants were obtained by centrifugation of the cultures and filtration of the supernatants through a Millipore filter, 0.45 μ. Supernatants were assayed for lymphotoxin by a modification of the method of Kolb and Granger (9). Indicator L cells in Eagle's minimum essential medium with Earle's salts (EMEM) and 10% fetal calf serum were established 24 hr prior to use. Cell concentration was adjusted to 9×10^4 cells/ml and 1.0 ml was added to 16 $\times$ 150-mm glass or plastic screw-capped tubes. Following incubation, each tube was examined microscopically and tubes with nonuniform monolayers were eliminated. Growth medium was removed and replaced with 1.5 ml of test medium, an aliquot of lymphocyte supernatant diluted in EMEM supplemented with glutamine (final conc 2 mM), and fetal calf serum to maintain a final concentration of 10%. Cultures were incubated at 36° in an atmosphere of 95% air and 5% CO_2. At intervals, the cultures were observed microscopically for evidence of CPE. Loss of cell viability was determined by measuring the capacity of cultures to incorporate ^{14}C amino acids into cellular protein. Test medium was removed and replaced with 1.5 ml of ^{14}C amino acid

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labeling medium (MEM, Hanks' salts, containing 1:20 amino acids and ^{14}C amino acids at a concentration of 0.1 μCi/ml). The entire procedure was carried out at 36° using prewarmed reagents. Following incubation for 30 min, the cultures were chilled in ice and then processed for counting according to Kolb and Granger (9). Samples in 15 ml of a solution of 0.5% 2,5-diphenyloxazole (PPO) and 0.03% 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (dimethyl POPOP) in toluene, were counted in a Tri-Carb liquid scintillation spectrometer (Packard Instrument Company, Inc., Downers Grove, Illinois). Cell cultures and tissue culture reagents were obtained from Grand Island Biological Company. ^{14}C amino acids (15 amino acids, 100 μCi/ml) were purchased from New England Nuclear Corp., Boston, Mass.

Results and Discussion. Peripheral blood lymphocytes from noncancer patients and patients with cancer other than Hodgkin's disease were stimulated to release a cytotoxic factor following *in vitro* exposure to phytohemagglutinin (Fig. 1). This was indicated by a decrease in uptake of ^{14}C amino acids by mouse fibroblast cultures following the addition of supernatants from human lymphocytes exposed to phytohemagglutinin. There was a marked decrease in the ability of supernatants from phytohemagglutinin-exposed peripheral blood lymphocyte cultures from patients with Hodgkin's disease to produce a similar cytotoxic effect (T-distribution analysis revealed >99.5% significance). Figure 2 shows the effects of supernatants from control cultures of noncancer, cancer, and Hodgkin's disease patients on the mouse fibroblast test system. These effects were compared at 24 and 48 hr. In L cell cultures incubated with supernatants from lymphocytes of noncancer and cancer patients, a progressive uptake of radioactive isotope was observed. A similar progressive uptake of radioactive isotope was not observed in cultures of L cells incubated with supernatants from lymphocytes of patients with Hodgkin's disease (T-distribution analysis revealed a similar >99.5% significance). The implication of the above finding is that supernatants of control peripheral blood

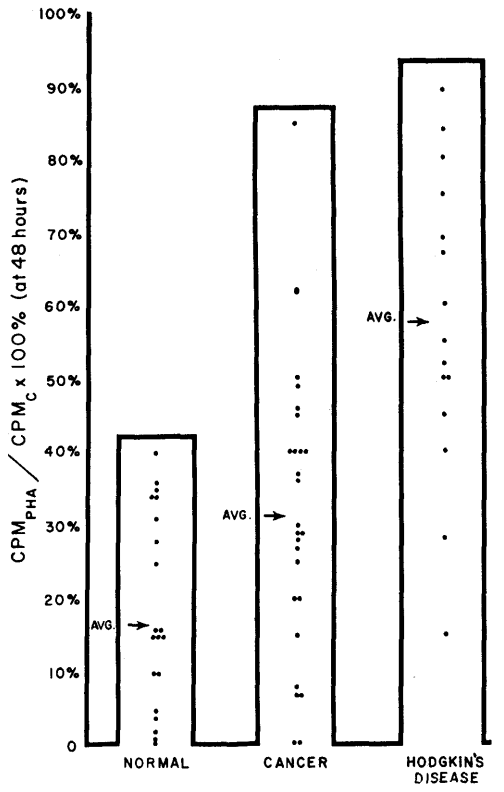

FIG. 1. Assay of lymphotoxin in supernatant fluid from 5-day peripheral blood lymphocyte cultures from non-cancer patients, patients with cancer, and patients with Hodgkin's disease as measured by inhibition of ^{14}C amino acid uptake by mouse L cells.

lymphocyte cultures from patients with Hodgkin's disease which have not been exposed to phytohemagglutinin contain a material which has a lymphotoxin-like effect on mouse fibroblasts. This material was not detected in unstimulated cultures of lymphocytes from noncancer patients or patients who had cancer other than Hodgkin's disease. The lymphocytes of Hodgkin's disease patients produced less lymphotoxin following exposure to phytohemagglutinin than did the lymphocytes of noncancer patients or patients with cancer other than Hodgkin's disease. It appears that while the peripheral blood lymphocytes of pateints with Hodgkin's disease have a decreased capacity to release lymphotoxin following exposure to phytohemagglutinin, they also have the capacity for spontaneous release of a

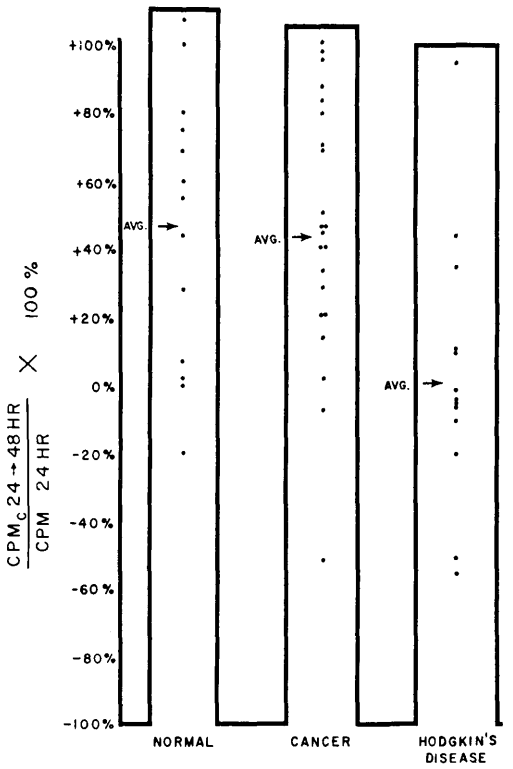

FIG. 2. Effect of 5-day unstimulated peripheral blood lymphocyte culture supernatant fluid from non-cancer patients, patients with cancer, and patients with Hodgkin's disease upon the change in ^{14}C amino acid uptake by mouse L cells at 24 and 48 hr.

lymphotoxin-like substance when unstimulated by phytohemagglutinin. It is possible that the lymphotoxin-like substance spontaneously released by the Hodgkin's disease lymphocytes is a substance similar to the lymphotoxin which is released by other lymphocytes only upon stimulation. Should this lymphotoxin-like substance have the same physical and chemical properties as lymphotoxin, it would then be apparent that the Hodgkin's disease lymphocyte, while able to produce lymphotoxin under unstimulated conditions, is at the same time unable to augment this degree of lymphotoxin production following stimulation. This situation could be analogous to the spontaneous production of fixed amounts of antibody that occurs in patients with multiple myeloma, and the release of lymphotoxin by the Hodgkin's disease lymphocytes may represent either a host defense response to an antigenic stimulus or a fixed neoplastic release of lymphotoxin.

Summary. The *in vitro* release of lymphotoxin by human peripheral blood lymphocytes from noncancer patients, patients with cancer, and patients with Hodgkin's disease were studied. Noncancer patients and patients with cancer other than Hodgkin's disease released no lymphotoxin under unstimulated conditions while stimulation with phytohemagglutinin induced *in vitro* release of lymphotoxin. Lymphocytes from patients with Hodgkin's disease released significantly less lymphotoxin following stimulation with phytohemagglutinin but also released measurable amounts of a lymphotoxin-like substance in the unstimulated state. Hodgkin's disease lymphocytes appear to differ from the lymphocytes of noncancer patients and patients with cancer other than Hodgkin's disease by both lower *in vitro* lymphotoxin release following phytohemagglutinin stimulation and spontaneous release of lymphotoxin by unstimulated lymphocytes.

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