Vol. 88, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
June 13, 1979 Pages 804-809

A NOVEL EARLY EFFECT OF CONCANAVALIN A ON THYMOCYTES

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Received April 6,1979

SUMMARY: Concanavalin A, added to freshly isolated rabbit thymocytes, markedly enhanced the extracellular appearance of non-immunoglobulin proteins. Time course studies revealed that the onset of enhancement occurred virtually without delay. The effect appeared to be restricted only to certain of the thymus-derived cells because thymocytes obtained from rabbits treated with hydrocortisone, as well as splenocytes derived from untreated rabbits essentially did not exhibit the enhancement. Stimulation by concanavalin A was specific in that pokeweed mitogen and lipopolysaccharide were without effect and also in that α-methyl-mannoside, but not galactose, abrogated the concanavalin A-mediated enhancement. Experiments with mouse thymocytes demonstrated that the cells which responded to concanavalin A were primarily cells that bear the θ-antigen on their surface (T-cells).

Differentiation of precursor lymphoid cells can be initiated by a number of agents including the lectin concanavalin A (Con A). Aside from its effect on blast cell formation and on cell proliferation, Con A is known to induce a wide variety of biochemical changes in lymphocytes. Thus, it has been reported to cause enhanced uptake of extracellular nutrients (1-3) and to result in increased synthesis of RNA (4), DNA (5), fatty acids (6) and proteins (7). Con A produces changes in the cellular concentration of cyclic nucleotides (8) and also induces the extracellular appearance of lymphokines which are capable either of inhibiting (9) or enhancing (10) the synthesis and secretion of immunoglobulins.

The possible correlation between these biochemical events is complicated by the fact that some of the observed changes occur relatively rapidly while others occur only after a considerable delay. For example, Ca^{++} influx occurs in a matter of seconds (1), RNA synthesis occurs in a

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matter of hours and lymphokine production, DNA synthesis and cell proliferation occur in a matter of a day or longer (11).

In the process of studying the effect of Con A on the secretion of [^{3}H]-proteins by thymocytes, we observed that Con A, even when added to freshly isolated cells, induced a marked enhancement in the extracellular appearance of [^{3}H]-proteins other than [^{3}H]-immunoglobulins and that such [^{3}H]-proteins appeared essentially without delay. This novel activity of Con A is the subject of this report.

MATERIALS AND METHODS

Cell suspensions were prepared from thymuses of New Zealand white rabbits (approximately 2.0 kg) by teasing the tissue apart with wire brushes as previously described (12). The cells were collected in medium 199 containing 10 percent fetal calf serum, penicillin and HEPES buffer, pH 7.5 (13-15). After washing with leucine-free medium 199 containing 0.02 M HEPES, pH 7.5, the cells were incubated at 37° in this same medium supplemented with [^{3}H]-leucine (5.0 Ci/mmole) and also, where indicated, with Con A at a final concentration of 10 µg/ml. At the end of the incubation period the cells were sedimented by centrifugation (600 x g - 10 min) and discarded. To insure removal of [^{3}H]-immunoglobulins, carrier rabbit immunoglobulins and antiserum specific for rabbit immunoglobulins were then added to the extracellular fluid. The mixture was incubated for 1 hr at 37° followed by 18 hours at 3° prior to removal of the precipitate. This procedure was routinely employed to permit unambiguous comparisons of thymocytes with other lymphoid cells which may secrete substantial quantities of immunoglobulin. The [^{3}H]-leucine-labeled materials in an aliquot of the immunoglobulin-free extracellular fluid were precipitated with TCA at a final concentration of 5 percent. The resultant precipitate was washed twice with TCA, heated in the presence of TCA at 90° for 15 min and finally dissolved by boiling for 1.5 min in the presence of 2 percent sodium dodecyl sulfate prior to determination of the radioactivity using Hydromix (Yorktown Research, S. Hackensack, NJ) scintillation fluid.

Cortisone-resistant thymocytes were prepared from rabbits injected i.p. (125 mg/kg) 48 hours prior to removal of the thymus. Antibody with specificity for rabbit immunoglobulins was prepared in goats, as previously described (12).

[^{3}H]-leucine was obtained from New England Nuclear, Boston, MA; medium 199 and fetal calf serum were obtained from Grand Island Biologicals, Grand Island, NY. Rabbit immunoglobulins were purchased from Pentax, Kankakee, IL, and anti-Θ antiserum from Accurate Chemical and Scientific Corporation, Hicksville, NY. Hydrocortisone acetate was obtained from Sigma Chemical Company, St. Louis, MO and Biogel A-0.5 from Bio-Rad Laboratories, Richmond, CA.

RESULTS

A time course for the extracellular appearance of [^{3}H]-proteins, derived from thymocytes that had been incubated with or without Con A,

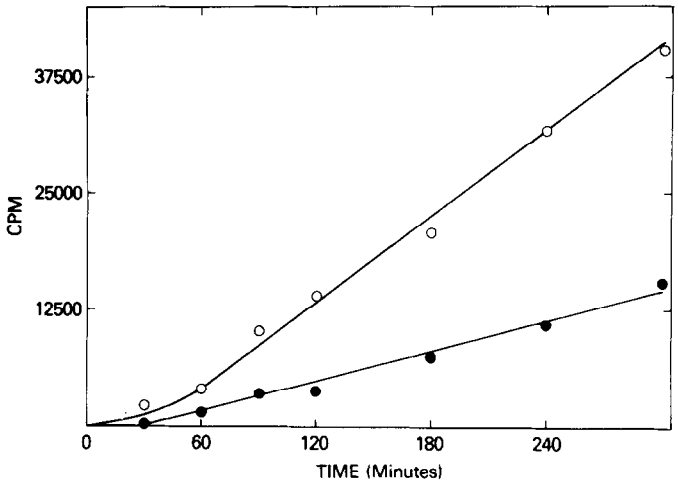

Fig. 1 - Time course of extracellular appearance of [^{3}H]-proteins. Thymus cells (8 x 10^7) in 1.0 ml of leucine-free medium 199 supplemented with 10 µCi of [^{3}H]-leucine were incubated with or without 10 µg of Con A in 17 x 100 mm plastic tubes. The tubes were incubated at 37° with shaking (70 rpm) and centrifuged (600 x g - 10 mins) at the indicated time to remove cells. The supernatant fluids were rendered free of [^{3}H]-immunoglobulins and processed as described in Materials and Methods. Open circles = with Con A, closed circles = without Con A.

is shown in Fig. 1. In the absence of Con A, the [^{3}H]-proteins first appeared at about 30 minutes and then increased linearly through the first five hours. On the other hand, in the presence of Con A, a slight stimulation was detected at 30 minutes and thereafter the appearance of [^{3}H]-proteins was markedly increased over the control. Although phytohemagglutinin mimicked the stimulatory activity of Con A, neither pokeweed mitogen nor lipopolysaccharide induced enhanced extracellular appearance of [^{3}H]-proteins (data not shown). That Con A was functioning via its site known to bind carbohydrate was established by the finding that α-methyl-mannoside, but not galactose, inhibited the Con A-mediated stimulation.

Chromatographic analysis of the Con A-enhanced extracellular [^{3}H]-proteins on Biogel A-0.5 revealed that the proteins eluted mainly in an inhomogeneous band with a peak in the region of 135 ml (Fig. 2-A). The remainder of the elution pattern was indistinguishable from the pattern exhibited by the control extracellular [^{3}H]-proteins. In contrast to

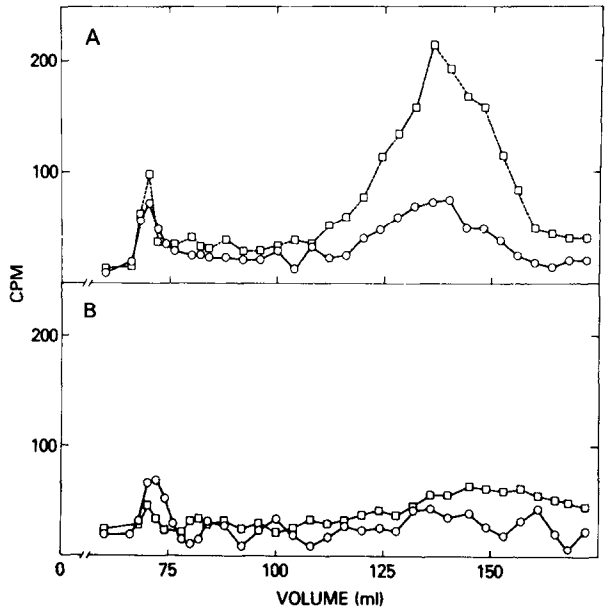

Fig. 2 - Chromatography of the extracellular product on Biogel A 0.5 m. Thymus cells (4×10^8) in 5.0 ml of leucine-free medium 199 supplemented with 50 µCi of [^{3}H]-leucine were incubated with Con A (50 µg) or without Con A for 2 hours, in 50 ml tissue cluture flasks. Cells were removed by centrifugation (600 x g - 10 min), the extracellular fluids concentrated by vacuum dialysis in collodion sacs and dialyzed further with 0.01 M phosphate pH 7.4 - 0.15 M NaCl (buffered saline). Carrier rabbit immunoglobulins and goat anti-rabbit immunoglobulins were added and incubated for one hour at 37°. After removal of the immune precipitate, the supernatant fluid was applied to the Biogel column (93 x 1.6 cm) previously equilibrated with buffered saline, and the column then developed with buffered saline. Open circles = without Con A, open squares = with Con A. Panel A = normal thymus cells, Panel B = thymocytes from cortisone-treated rabbits.

these results, thymus cells derived from rabbits previously treated with hydrocortisone, known to deplete the thymus of certain subpopulations (16), failed to exhibit the Con A-mediated enhancement (Fig. 2-B). Furthermore, splenocyte populations from untreated rabbits did not exhibit the Con A enhancement that was readily observed with autologous thymocytes.

To assess whether or not the increase in extracellular [^{3}H]-protein was a consequence of cell death, we examined the possible toxicity of Con A on thymus cells with regard to cell viability using the trypan blue, dye exclusion test (17). The possibility of lysis or leakage of

intracelullar protein was measured by determining the extracellular appearance of lactic dehydrogenase (18). In one out of three experiments, a two-fold increase in thymocyte death and/or leakage due to Con A was observed. Of the remaining two experiments, thymocytes incubated with or without Con A yielded identical results in regard both to the dye exclusion and lactic dehydrogenase assay. In comparison to these findings, Con A induced an increase in extracellular [^{3}H]-protein in every case. Thus, of eleven experiments, an average increase of 3-4-fold over the control was observed.

The Con A-mediated enhancement observed with rabbit thymocytes was also observed using mouse thymus cells. When mouse thymocytes were treated with T-cell specific antiserum (anti-θ) plus complement before incubation with Con A, more than 85 percent of the enhancement was abolished.

DISCUSSION

The results demonstrate that the addition of Con A to thymocytes causes a rapid release of [^{3}H]-leucine labeled proteins to the extracellular medium. The mechanism by which the cells release [^{3}H]-protein is not known and may be due either to secretion, or alternatively to the shedding of membrane proteins (19,20).

Since the release of [^{3}H]-protein in response to Con A occurs relatively rapidly, it is not unreasonable to consider the possibility that the relased product may act, in turn, as a lymphokine and thereby regulate or induce, in part, the biochemical changes that occur subsequent to Con A addition. While this possibility is quite speculative, it is also attractive in that it is amenable to experimental test.

ACKNOWLEDGEMENT

The excellent technical assistance of Mrs. Blossom Johnson is gratefully acknowledged.

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