Phytohemagglutinin-Induced Cytotoxic Action of Normal Lymphocytes on Cells in Tissue Culture Using $^{14}$C-Leucine Incorporation

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NEMOTO, R., ISHIKAWA, K. and KATO, T. Phytohemagglutinin-Induced Cytotoxic Action of Normal Lymphocytes on Cells in Tissue Culture Using $^{14}$C-Leucine Incorporation. Tohoku J. exp. Med., 1977, 123 (4), 389-391 — Stimulation of normal peripheral blood lymphocytes (PBL) by phytohemagglutinin (PHA) and production of PHA-induced cytotoxicity were investigated. PBL transformation stimulated by PHA was measured by $^3$H-thymidine incorporation, and the cytotoxicity was assayed by measuring the reduction of $^{14}$C-leucine incorporation into target cells after interaction with PHA-treated PBL. The cytotoxicity of PHA-treated PBL showed dose response curves which corresponded with that of PBL transformation. Reduced $^{14}$C-leucine incorporation of target cells caused by cytotoxic PBL would be a reliable measure of immune reactions. —— $^{14}$C-leucine; lymphocyte; PHA-induced cytotoxicity

There are a variety of methods for assessment of the interaction between peripheral blood lymphocytes (PBL) and target cells in vitro. The most common method is based upon microscopic observation of surviving target cells (Takasugi and Klein 1970). Previously we reported that $^{14}$C-leucine incorporation into cultured cells correlated well with microscopic counting of surviving cells and could be used as a measure of cell viability (Kato et al, 1977b). In the present paper, we describe that the $^{14}$C-leucine incorporation into monolayer culture can be employed in evaluation of cell damage in immune reactions.

**Materials and Methods**

Preparation of PBL. Approximately 20 ml of heparinized peripheral blood lymphocytes (PBL) from healthy volunteers were separated by the Ficoll-Contrary gradient technique (Boyum 1968) and washed three times with phosphate buffer solution (PBS).

Incubation of PBL. PBL were divided into two components, one of which was used as effector cells in cytotoxicity test at a concentration of $10^7$ cells/culture, and the other for the determination of PBL transformation by phytohemagglutinin (PHA) at $10^4$ cells/culture. Various concentrations of PHA-P (New England Nuclear Co.) were added at the start of the culture suspended in Eagle's MEM medium (EM) supplemented with 10% fetal calf serum.

For the determination of lymphocyte transformation by PHA, PBL were incubated for 48 hr, followed by labeling with $1 \mu$Ci/ml of $^3$H-thymidine (New England Nuclear Co.) for

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24 hr. After incubation the PBL were washed twice with cold saline and extracted with 5% trichloroacetic acid. The precipitate was lysed by addition of 0.5 N NaOH. The lysate was solubilized with PCS scintillator (Amersham/Searle Co.) and radioactivity was measured by a scintillation counter (Nuclear Chicago Co.). PBL response to PHA was calculated from the following equation: response index = cpm in presence of PHA/cpm in absence of PHA.

**Tumor cells.** T24 cells established from human bladder carcinoma (Bubenik et al. 1973; Kato et al. 1977a) were used as target cells. The cell line was maintained in EM with 10% fetal calf serum.

**Cytotoxicity assay.** 5 x 10⁴ target cells were cultured in flat-bottom culture tubes (15 mm in diameter). After 48 hr incubation the medium and non-adherent cells were discarded. 5 x 10⁴ PBL in 1 ml of EM, which had been incubated in the various concentrations of PHA for 72 hr, were seeded into the tubes. At the end of 48 hr incubation the cultures were swirled to suspend the PBL, the suspender containing PBL as well as detached cells were discarded, and the attached cells were washed with PBS. The cells were allowed to recover for next 24 hr in fresh medium and labeled with 0.5 μCi/ml of ¹⁴C-leucine (New England Nuclear Co.) for 24 hr. The cells were harvested and the content of ¹⁴C-leucine was measured as described previously (Kato et al. 1977b). The data indicated were average cpm from 4 test samples. Extent of target cell death was calculated from the following equation: % target cell died = 100 - (cpm in target and effector cells/cpm in target cells) x 100.

**RESULTS AND DISCUSSION**

Fig. 1 shows the incorporation of ¹⁴C-leucine into the target cells after incubation with PBL pretreated by various concentrations of PHA. At concentrations of PHA higher than 30 μg/ml, the incorporation of ¹⁴C-leucine decreased significantly, which indicated decreased viability of target cells following the interaction with PHA-treated PBL (Kato et al. 1977b). Fig. 2 shows the effect of PHA on cyto-

![Graph](image-url)

**Fig. 1.** ¹⁴C-Leucine incorporation (cpm, mean±s.d.) into the target cells after incubation with normal lymphocytes pretreated with various concentrations of PHA. C, cells incubated without lymphocytes.
Fig. 2. Effect of concentration of PHA on cytotoxicity and stimulation of lymphocyte. ○, percentages of target cells died; ●, response index of lymphocyte to PHA (mean ± s.d.)

toxicity and stimulation of PBL. Stimulation of DNA synthesis in PHA-treated PBL followed a dose response curve which was similar to that of cytotoxic action against the target cells. These findings agreed to the previously reported data (Holm and Perlmann 1967).

The results of the present study indicate that the 14C-leucine incorporation into target cells can be used as a sensitive and simple method for the determination of the cell-mediated cytotoxicity.

References