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ENHANCEMENT OF β_2-MICROGLOBULIN FORMATION INDUCED BY PHYTOHEMAGGLUTININ AND MERCURIC ION IN CULTURED HUMAN LEUCOCYTES

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SUMMARY

Human leucocyte cell cultures were stimulated to initiate DNA synthesis by phytohemagglutinin and mercuric chloride. Both mitogens enhanced the accumulation of β_2-microglobulin in the medium, which was synthesized by lymphocytes. Mercuric chloride promoted the accumulation of this protein optimaly with a concentration (1×10^{-5}M) to produce the maximum stimulation of DNA synthesis. Combined use of phytohemagglutinin (50 µg/ml) and mercuric chloride (1×10^{-5}M) produced additive effect on both DNA synthesis and β_2-microglobulin accumulation. These findings suggest that mercuric ion causes the proliferative response of lymphocytes by a mechanism different from that for the stimulation by phytohemagglutinin.

INTRODUCTION

Transformation of human lymphocytes is well known to be induced *in vitro* by phytohemagglutinin (PHA) (1). Some heavy metal ions have been shown to stimulate lymphocytes as a non-specific mitogen like PHA. Normal human lymphocytes can enhance DNA synthesis and transformation in response to heavy metal ions such as mercury (2-4), nickel (4,5) and zinc (4,6,7). Among those heavy metal ions, mercuric ion has been the most potent stimulant (4).

β_2-Microglobulin is a simple protein of a molecular weight 11,800 and has been located on the cell surface of lymphocytes (8). Its synthesis and secretion by lymphocytes *in vitro* have been found (9) and promoted under the stimulation of PHA (10,11). Moreover, antiserum or antibody of β_2-microglobulin inhibits the proliferative response of lymphocytes to PHA (12), the mixed lymphocyte reaction (12,13) and an antigen-induced proliferative response (13). These facts mean that this protein may play a critical role on the

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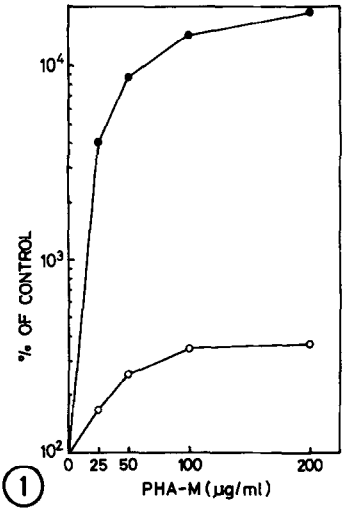
response of lymphocytes to mitogens and antigens. It has prompted us to investigate whether or not the accumulation of β_2-microglobulin by lymphocytes *in vitro* is enhanced by mercuric ion. The present work has shown that this metal ion increases the accumulation of β_2-microglobulin, but that it may stimulate lymphocytes by a mechanism differnt from that for the stimulation by PHA.

MATERIALS AND METHODS

Leucocytes were obtained from the peripheral blood of a normal donor with no history of sensitivity to mercury by allowing the blood to settle for 1 hour at 37°C after adding dextran to give a final concentration of 0.4%. Aliquots of the cell suspension containing approximately 10^6 cells per ml medium were cultured in 1 ml Eagle's minimum essential media containing 20% heat-inactivated fetal calf serum, 50 units/ml penicillin G and 50 µg/ml streptomycin under 5% CO_2 atmosphere. At the onset of cultivation, 20µl sterilized solution of PHA-M (Grand Island Biological Co., Grand Island, N.Y., U.S.A.) and/or mercuric chloride were added to appropriate cultures to give a final concentration of 12.5 to 200 µg per ml medium and 1×10^{-6} to 1×10^{-4}M, respectively. For the mercury experiment two kinds of control cultures were set up: one with 50 µg PHA per ml medium and another one without any stimulant. Lymphocyte stimulation activity was estimated by determining [^{3}H]thymidine incorporation into cold trichloroacetic acid-insoluble (acid-insoluble) fraction of cells. Tritiated thymidine (0.5 µC/ml culture, specific activity 150 mC/m mol) was added for the final 24 hours of the culture by use of 20 µl of working solution. DNA synthesis was then arrested by the addition of 1 ml ice-cold saline and immersing in ice bath. The cells was separated by centrifugation at 50g for 5 min from the medium which was collected for β_2-microglobulin assay. The precipitated cells was re-suspended in saline and was then filtered on the glass fiber filter discs (Whatman GF/C) with a sampling manifold (Millipore Co., Ltd.). Those discs were washed twice with ice-cold 5% trichloroacetic acid and once with ice-cold ethanol. Tritium radioactivity on the disc was counted with a liquid scintillation system. Amounts of β_2-microglobulin in the media were determined by a radioimmunoassay method (11, 14).

RESULTS AND DISCUSSION

Figure 1 shows the dose-effect relationship for lymphocyte stimulation and β_2-microglobulin accumulation in the medium after 3-day culture. PHA caused a significant increase in tritiated thymidine incorporation into acid-insoluble fraction of cultured leucocytes under the presence of 12.5 to 200 µg PHA-M per ml medium. Amounts of β_2-microglobulin in the medium increased from 41 to 150 ng/ml medium in response to the increased concentration of PHA. Human β_2-microglobulin was not detected in the original medium before culturing. As cells were maintained under healthy condition after 3-day culture, the increased accumulation of β_2-microglobulin in the medium seems to result



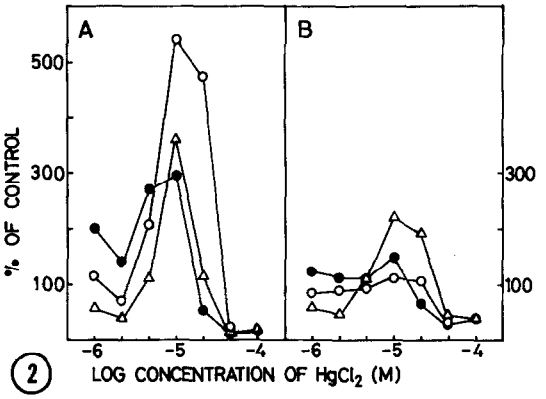


Fig. 1. Dose-effect curves of PHA-M for DNA synthesis and β_2-microglobulin accumulation in the medium by cultured leucocytes after 3-day culture. Closed circles represent percentage of the control for tritiated thymidine incorporation into ice-cold trichloroacetic acid insoluble fraction of cells during 24 hrs: open circles represent that for β_2-microglobulin accumulation in the medium during 3 days.

Fig. 2. Dose-effect curves of mercuric chloride for DNA synthesis during 24 hrs (2A) and β_2-microglobulin accumulation in the medium during 6 days (2B) by cultured leucocytes after 6-day culture. Results from three separate experiments are shown.

from the enhanced formation of this protein in the cells. In a preliminary experiment only less than 1% amount of β_2-microglobulin in the medium was found in the sonicated cells. This indicates that most of β_2-microglobulin formed by cells is actively excreted out of cells.

Mercuric chloride also caused enhancement of tritiated thymidine incorporation into acid-insoluble fraction of cells after 6-day culture (Fig. 2A). The maximum stimulation of the labeled thymidine incorporation by mercuric ion was 3 to 6 folds of controls at the concentration of 1×10^{-5}M, although it was much less than that by PHA. Mercuric ion did not stimulated the cultured cells at 3-day culture. Figure 2B shows that the accumulation of β_2-microglobulin in the medium was promoted by addition of mercuric ion. The maximum accumulation of β_2-microglobulin in the medium (64 to 80 ng/ml medium) was detected at the corresponding concentration of mercuric chloride with which the maximum stimulation of the labeled thymidine incorporation was

obtained. Mercuric ion seemed not to promote the cell disruption with concentrations below 2.5 x 10^{-5}M, because cell counts per culture after 6-day culture were not differnt significantly between control and mercury-treated cultures. At concentrations more than 2.5 x 10^{-5}M, mercuric ion was apparently toxic to the cells and decreased both cell counts per culture and β_2-microglobulin accumulation in the medium. These findings mean that active accumulation of β_2-microglobulin in the medium is mainly due to the enhanced formation of this protein by viable cells, although the alteration in degradation of β_2-microglobulin has not been studied yet. Enhancement of β_2-microglobulin formation may be one of proliferative responses of lymphocytes to mercuric ion such as blastogenesis and mitosis (3,15). The similarity between dose-effect curves of PHA or mercuric ion for the labeled thymidine incorporation and β_2-microglobulin accumulation suggests that there seems to be a close correlation between lymphocyte stimulation and active formation of β_2-microglobulin by the cells.

Figure 3A and 3B show the effect of mercuric ion on the action of PHA to the labeled thymidine incorporation and β_2-microglobulin accumulation in the medium after 6-day culture. Mercuric chloride was added simultaneously with PHA at the onset of the culture. The optimaly stimulating concentration of mercuric chloride (1 x 10^{-5}M) potentiated the stimulating action of PHA on both the labeled thymidine incorporation and β_2-microglobulin accumulation in the medium. Inhibitory concentrations of mercuric chloride (2.5 x 10^{-5}M and 5 x 10^{-5}M) manifestly depressed the augmentation of both indicators by PHA. The additive effect of mercuric chloride and PHA indicates that a mechanism differnt from that for the proliferative response to PHA should be considered for the response to mercuric chloride. It is probable that mercuric ion may act on a different site of the cell surface or a differnt population of lymphocytes from that for PHA. Recently it has been reported that at 3-day culture mercuric chloride blocks PHA stimulation of mouse lymphocytes (16) and that organic mercuric compounds reduce PHA stimulation of human lymphocytes

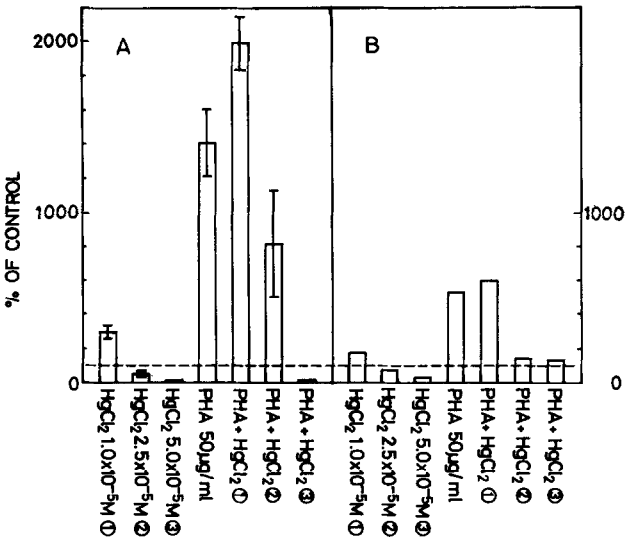


Fig. 3. Effect of mercuric chloride on the stimulating action of PHA to DNA synthesis during 24 hrs (3A) and β_2-microglobulin accumulation in the medium during 6 days (3B) by cultured leucocytes after 6-day culture. Vertical lines in Figure 3A mean standard deviations. PHA and mercuric chloride were added simultaneously to the cultures at the onset of the culture. Broken lines represent the control level (100%).

(17). The optimal stimulation appears approximately on the 3rd day of the culture for PHA and on the 6th day for mercuric chloride (3). The differnt effect of mercuric chloride on PHA stimulation of lymphocytes may be due partly to the differnt culture duration used for experiments. It remains for further studies whether the addition of mitogens increases the synthesis of β_2-microglobulin per cell or the number of β_2-microglobulin-producing cells.

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