Cytochalasin B Enhances T Cell Mitogenesis by Promoting Expression of an Interleukin 2 Receptor

Hiroshi Komada*, Hiroshi Nakabayashi, Mari Idota, Masayuki Hara**, Takao Takahashi**, Hideki Takanari and Kosaku Izutsu

Department of Pathology, *Department of Microbiology, Mie University School of Medicine and **Department of Agricultural Chemistry, Faculty of Agriculture, Tsu, Mie 514, Japan

ABSTRACT. Low concentrations of cytochalasin B enhanced the T cell mitogenesis induced by concanavalin A (Con A) and interleukin 2 (IL-2). Mitogenesis was augmented by cytochalasin B given in the Con A-dependent early phase, or through T cell mitogenesis. Cytochalasin B did not enhance T cell mitogenesis when given only in the IL-2-dependent late phase. Use of the monoclonal antibody that directs the IL-2 receptor showed that cytochalasin B increased the expression of the IL-2 receptor induced by Con A. We concluded that cytochalasin B acts on an early phase of T cell mitogenesis and augments the expression of IL-2 receptor which enables certain nonresponsive T cells to respond to IL-2.

Larsson et al. have shown that, in a relatively short time, the binding of Con A to purified T cells modifies their functional sensitivity to growth factors (9). We have reported that Con A initiates T cell mitogenesis and that IL-2 stimulates T cells shortly before the onset of DNA synthesis (7). T cell transformation can be divided into two stages; a Con A-dependent initial half stage and an IL-2-dependent last half stage. Low concentrations of cytochalasin B triggered the response of lymphocytes to mitogens (4, 5, 11, 13). Cytochalasin B, a fungal product, affects various cell motilities, such as microfilament assembly and cell morphology (1, 12). Augmentation of lymphocyte mitogenesis by cytochalasin B may result from its effect on the cytoskeleton and intracellular biochemical events (2, 3, 4).

We here present the effect of cytochalasin B on the T cell mitogenesis induced by Con A and IL-2. The stage at which the cytochalasin B effects take place and the effect of cytochalasin B on the expression of the IL-2 receptor are discussed.

MATERIALS AND METHODS

Human lymphocytes were obtained from resected tonsils of children 4- to 12-years old with Ficoll paque (6, 10). T lymphocytes were prepared from human tonsillar lymphocytes with a nylon wool column as described elsewhere (7). About 80% of the cells were T cells and about 20% B cells obtained with monoclonal antibodies (B1, T4 and T8, Coulter Immunology, U.S.A.). More than 90% of the cells formed rosettes with sheep red blood cells. The T cells were cultured at 1 x 10^6 cells/ml in TC199 medium (Nakarai Chemicals Ltd.) supplemented with 20% calf serum (Nakarai Chemicals Ltd.) in a 24-well microplate in
triplicate cultures. Con A (Calbiochem), IL-2 (Electro-Nucleonics, Inc.) and various amounts of cytochalasin B (Funakoshi) were added, and culture continued for 72 h. For the last 3 h, 3 µCi [methyl-3H]thymidine (Radiochemical Centre, Amersham) was added. The radioactivity was counted as described elsewhere (6, 10). The stimulation index was given as the ratio of [methyl-3H]thymidine incorporation (cpm) induced by Con A plus IL-2 plus cytochalasin B to the incorporation induced by Con A plus IL-2. The expression of IL-2 receptor was determined with monoclonal antibody that directs the IL-2 receptor (Becton Dickinson) at 40 h of stimulation when there was maximum expression of the IL-2 receptor (8). The cells were stained by indirect immunofluorescence using anti mouse IgG goat IgG-FITC (Japan Immunoresearch Laboratories Co., Ltd.) then counted under a fluorescence microscope (Karl Zeiss, MPM 03).

RESULTS

Human T cells could be induced to synthesize DNA when given a low dose of Con A (10 µg/ml) together with IL-2 (10 µl/ml), but 10 µg/ml Con A alone did not induce T cell mitogenesis. The following experiments used these combined doses of Con A and IL-2. The cytochalasin B was not mitogenic in itself.

At relatively low concentrations (0.1 to 3 µg/ml), cytochalasin B enhanced Con A-
and IL-2-induced T cell proliferation, whereas high doses of cytochalasin B inhibited T cell mitogenesis (Fig. 1.a). When Con A and various doses of cytochalasin B were added to T cells at initiation and IL-2 was added 15 h after initiation, the maximum augmentation of DNA synthesis took place at 0.3 μg/ml cytochalasin B (Fig. 1.b). There was no increase in T cell proliferation when Con A and IL-2 were added at the beginning, followed by various amounts of cytochalasin B 15 h after stimulation (Fig. 1.c). These results indicate that low concentrations of cytochalasin B have an auxiliary effect in the early phase of T cell mitogenesis and that cytochalasin B is not a co-stimulator of IL-2. The following examination was designed to confirm these results. Various concentrations of cytochalasin B were added at the time of the Con A additions. Cells were washed with 0.1 M α-methyl mannanside (α-MM) at 15 h of Con A stimulation after which IL-2 was added, then DNA synthesis was determined at 72 h (Fig. 2.a). Cytochalasin B given only during the initial phase in T cell mitogenesis enhanced T cell proliferation. We concluded that cytochalasin B does trigger the response of T cells to Con A in the Con A-dependent early process and does make T cells respond to IL-2. When 50 μg/ml of Con A was added to T cells that then were washed with α-MM at 15 h, and IL-2 added, DNA synthesis took place (7). Therefore, 50 μg/ml of Con A was added to T cells to initiate mitogenesis. The cells then were washed with α-MM at 15 h, after which IL-2 and cytochalasin B
were added and DNA synthesis determined (Fig. 2.b). Cytochalasin B did not enhance T cell mitogenesis when added after 15 h of stimulation. The effect of cytochalasin B on the induction of the expression of the IL-2 receptor by Con A was determined using monoclonal antibody that directs the IL-2 receptor. Cytochalasin B enhanced the low dose of Con A-induced expression of the IL-2 receptor as shown in Table 1.

### DISCUSSION

Several reports have shown that low concentrations of cytochalasin B (0.1 to 1 μg/ml) trigger the lymphocyte mitogenesis induced by phytomitogens (4, 5, 11, 13). There are no reports, however, on the effect of cytochalasin B on IL-2-induced lymphocyte proliferation. We here have shown that cytochalasin B affects the early stage of T cell mitogenesis and enhances the induction of the expression of IL-2 receptor. The potentiation of lymphocyte mitogenesis by cytochalasin B is not because of its action on microfilaments, but one another system because the concentration which causes enhancement of lymphocyte proliferation differs from that at which most reactions of the microfilaments occur (11). Cytochalasin B has been reported to enhance the early events of mitogenesis; i.e., amino acid uptake (4), phospholipid turnover (4), elevation of cyclic AMP (4), production of a 1.5- to 4.0-fold augmentation of the early increase in Ca²⁺ transport in response to PHA, Con A and periodate (3). Also, Ca²⁺, Mg²⁺ and a cytochalasin B-sensitive process are required for the lectin-dependent commitment to DNA synthesis (2). Hoffman et al. suggested that cytochalasin B affects cell-cell interaction, perhaps by affecting the distribution of Con A, or by increasing the response to chemotactic stimuli, and that cytochalasin B may act in some way that enables certain nonresponsive cells to respond to a mitogenic stimulus (5). The results of some researches support the supposition that cytochalasin B affects the Con A-dependent early process of T cell proliferation and that it augments the Con A-induced cell response to IL-2. We conclude that cytochalasin B is a co-stimulator of Con A; it promotes induction of the expression of the IL-2 receptor by Con A and enables nonresponsive cells to respond to IL-2.

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### REFERENCES

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