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AGE-RELATED DIFFERENTIAL EFFECTS OF ZINC ON CONCANAVALIN A-INDUCED CAPPING OF HUMAN LYMPHOCYTES

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INTRODUCTION

ZINC APPEARS to exert potent biological effects on a variety of cells. Zinc can act as a mitogen when added directly to lymphocyte cultures (Rühl *et al.*, 1971; Berger and Skinner, 1974). Zinc deficiency in animals has been associated with abnormal development of the thymus (Kroneman *et al.*, 1975), the lymphoid system (Tanaka *et al.*, 1978) and defective immune function (Iwata *et al.*, 1979; Fraker *et al.*, 1977; Fernandes *et al.*, 1979). Pharmacological doses of zinc suppress the sickling phenomenon in sickle cell anemia (Arnone and Williams, 1977). The mechanism of action of zinc, however, is not well understood. In the present paper I describe the effects of zinc on concanavalin A (con A)-induced capping in relation to age. The experimental evidence suggests that zinc might act by influencing the microfilament function and that alteration in microfilament function might be an important aspect of the aging phenomenon.

Interaction of ligands with surface receptors of lymphocytes leads to rapid changes in the surface membrane and polarization of the receptors leading to what is termed "cap" formation. Treatment of human peripheral blood lymphocytes (PBL) with fluoresceinated concanavalin A (F-con A) at 37°C induces cap formation in about 10% of the labeled lymphocytes. Cap formation can be altered by cytoskeletal modulating agents such as colchicine (de Petris, 1974; Albertini *et al.*, 1977) and cytochalasin B (CB) (de Petris, 1974; Yahara and Edelman, 1973). It has been proposed that the cell surface receptors are anchored to microtubules and microfilaments and disruption of these elements lead to alterations in surface receptor mobility (Yahara and Edelman, 1973).

Our previous work indicated that zinc modulates lectin-induced mitogenic responses of human PBL in an age-dependent fashion (Rao *et al.*, 1979). Further, we presented evidence indicating that the effect of zinc might be exerted through CB sensitive structures (Rao and Schwartz, 1980). This observation prompted me to study the effect of zinc on con A-induced cap formation of lymphocytes obtained from young and old individuals. The experimental evidence presented in this paper indicates that zinc has a differential ef-

fect on con A-induced lymphocyte capping, analogous to its effect on mitogenic responses (Rao *et al.*, 1979)

MATERIALS AND METHODS

Donors

Blood from young and aged healthy volunteer donors was freshly obtained from a peripheral vein. All donors gave informed consent consistent with the policies of The University of Michigan and the Department of Health and Human Services. The aging population ranged from 65–84 years; the young adult population ranged from 22–35 years.

Isolation of mononuclear cells

Mononuclear cells were prepared from fresh heparinized blood (20^{μ}/ml) by the Ficoll-Hypaque density gradient technique. The cells were washed three times with Ca^{++} and Mg^{++} free Hanks' balanced salt solution (HBSS, Grand Island Biological Co., Grand Island, NY) and resuspended in RPMI-1640 medium (Grand Island Biological Co.).

Drugs

CB and colchicine were obtained from Sigma Chemical Co., St. Louis, MO. Colchicine solution was prepared in water. CB was dissolved in 95% ethanol to a concentration of 2×10^{-3} *M* and appropriate dilutions were made in HBSS. $ZnCl_2$ was obtained from Fisher Scientific Co., NJ. F-Con A was obtained from Calbiochem-Behring Corp., LaJolla, CA.

Capping experiments

Mononuclear cells were suspended at a concentration of $4–6 \times 10^6$/ml, and 0.5 ml was used for each experiment. Drugs were added to the cells and the suspension was incubated at 37°C for 30 min with continuous rotation. Then F-con A was added to give a final concentration of 20 μg/ml and incubated for another 30 min. At the end of incubation the cells were fixed with 0.5 ml of 2% paraformaldehyde for 10 min, washed twice with HBSS and the number of caps formed were enumerated under fluorescent microscopy by counting 100 cells. Caps were defined as cells showing less than 1/3 surface fluorescence.

RESULTS

Cap formation with fluoresceinated con A

Fig. 1 illustrates the number of caps formed when the cells were treated with F-con A alone. The number of caps formed varied from 4%–16% and no difference could be detected in results obtained from cells from young and old donors (Student's *t*-test $p = 0.85$).

The monocyte content of our cell suspension did not seem to affect our results since many of the monocytes are lost (perhaps due to adherence to plastic) during the process of incubation at 37°C. Further, monocytes have a tendency to clump and during enumeration of capping under fluorescent microscopy clumped cells were omitted. In one experiment, where monocyte contamination was monitored with latex ingestion, few monocytes were detected at the time of counting, and the results were similar to non-latex ingested specimens. Thus, although a mononuclear cell preparation was used throughout these experiments the differences noted could be attributed mainly to lymphocytes.

Effect of colchicine on con A-induced capping

Preincubation with colchicine (10^{-5} *M*, final concentration) increased the number of

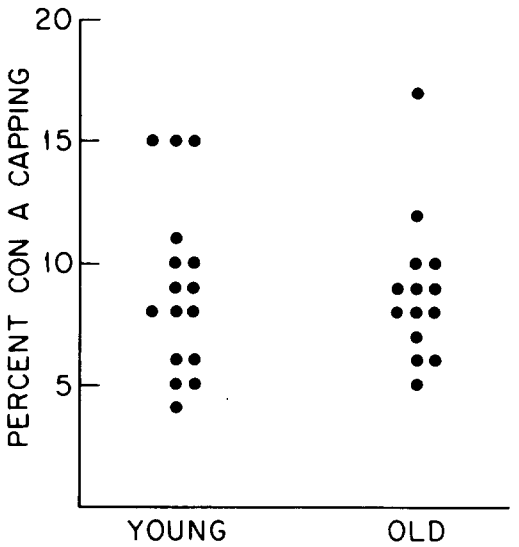

FIG. 1. Con A-induced capping of human peripheral blood lymphocytes. Lymphocytes were treated with 20 μg/ml (final concentration) of F-Con A and the number of caps formed were enumerated under fluorescent microscopy. Age of the young individuals was between 22–35 years and old individuals 65–84 years. In this and all subsequent figures each point represents data derived from a different cell donor.

caps formed in both groups (Fig. 2). The number of caps formed in the young population was 47 ± 15 (mean ± 1 SD), whereas in the old group it was 57 ± 9 (mean ± 1 SD). Statistical analysis by the Mann-Whitney used statistic test gave a value of 62.5 which was significant at $p = 0.024$.

Effect of colchicine plus zinc on con A-induced capping

Preincubation with zinc alone, followed by F-con A treatment failed to bring about a significant change in the number of caps formed as compared to treatment with F-con A alone (F-con A – 6%, zinc and F-con A – 5%). However, when mononuclear cells were incubated with both colchicine (10^{-5} *M*) and zinc (10^{-4} *M*) together for 30 min followed by F-con A treatment and compared with colchicine pre-treatment alone, dramatic difference was observed in the number of caps formed between the two groups (Fig. 3). Addition of zinc caused further enhancement of the number of caps formed in the younger age group as compared to the effect of colchicine alone, whereas in the aged population the number of caps decreased when compared to colchicine alone. The difference was highly significant ($p = 0.0002$) using the Wilcoxan Rank-Sum test.

Effect of colchicine plus cytochalasin B on con A-induced capping

The effect of CB on con A-induced capping was studied at two concentrations 10^{-6} *M* and 10^{-7} *M* (Fig. 4 a,b). At a concentration 10^{-6} *M* of CB (Fig. 4a) the number of caps formed decreased in both groups as compared to pretreatment with colchicine alone. At

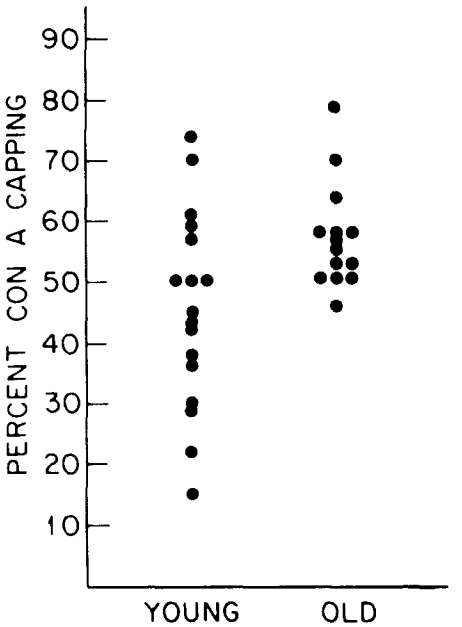

FIG. 2. Effect of colchicine on Con A-induced capping. Lymphocytes were pretreated with colchicine (10^{-5} *M*, final concentration) for 30 min and then incubated with F-con A for an additional 30 min.

10^{-7} *M* the effect was variable in both groups (Fig. 4b). Statistical analysis revealed no difference between the groups at these two concentrations of CB.

DISCUSSION

The experimental results show that treatment of human PBL with con A alone does not show any difference in the capping phenomenon between the young and aged population. This is in contrast to a recent report in which a difference in capping was noted between young and old individuals using con A alone (Naeim and Walford, 1980). This discrepancy could be due to the difference in the age of the older group which ranged from 80 to 98 in their study and 65 to 84 in my study. However, use of colchicine and zinc elicited significant differences between the young and old population in the present evaluation.

I offer the following interpretation for the observations described in this paper. It has been proposed that cell surface receptors are anchored to microtubules and microfilaments, and these structures have been attributed interrelated but opposite roles in maintaining the distribution and the mobility of surface receptors (Poste *et al.*, 1975 a,b). The inability of con A alone to demonstrate any difference between the two groups studied may be attributed to the anchoring effect of microtubules which might mask the differences in microfilament function. Once the microtubules are disrupted by colchicine, microfilaments, now unopposed, can bring about enhancement in capping and whatever differences exist between the microfilaments of lymphocytes from young and old persons become apparent. In these experiments when colchicine alone was used the differences

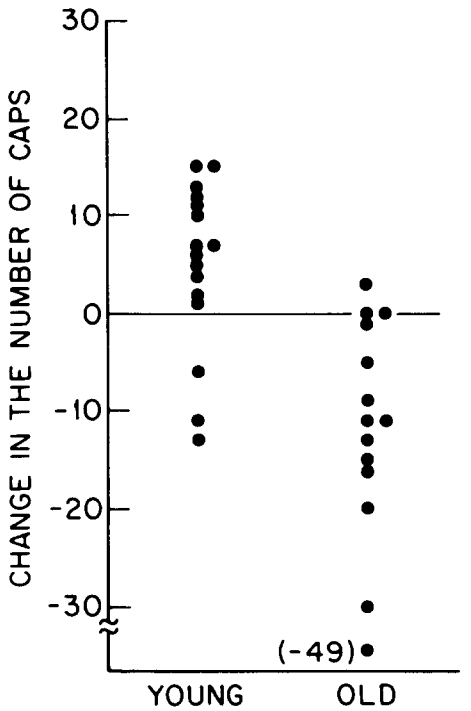

FIG. 3. Effect of colchicine plus zinc on con A-induced capping. Lymphocytes were pretreated with colchicine (10^{-5} *M*, final concentration) plus zinc (10^{-4} *M*, final concentration) for 30 min and then incubated with F-con A for an additional 30 min. The caps formed with colchicine plus zinc treatment were compared to colchicine treatment alone and the difference in the number of caps, per 100 cells counted, was plotted.

were significant at $p = 0.024$. But when zinc was added in addition to colchicine the difference was even more dramatic ($p = 0.0002$). Our previous observation that zinc modulates mitogenic responses by affecting CB sensitive structures (Rao and Schwartz, 1980) is consistent with the present finding that zinc is involved in modulating the capping phenomenon—another microfilament dependent function.

CB completely inhibits capping at high concentrations. For this reason the effect of CB on capping was tested at very low concentrations of CB; unlike zinc, CB does not show any age-dependent effects. This suggests that although both these agents act on microfilaments they might exert their action at different sites among the steps involved in microfilament formation or function. CB has been shown to inhibit actin polymerization by blocking filament elongation (Lin *et al.*, 1980). It is conceivable that zinc might be acting on some other molecule which might also have a role in microfilament formation or function. Calmodulin, a calcium regulating protein, is found in close association with microfilaments (Welsh *et al.*, 1978). It was proposed that the effect of zinc on cellular function might be mediated through its inhibitory action on calmodulin (Brewer *et al.*, 1979). Trifluoperazine, a known inhibitor of calmodulin (Levin and Weiss, 1977), can also in-

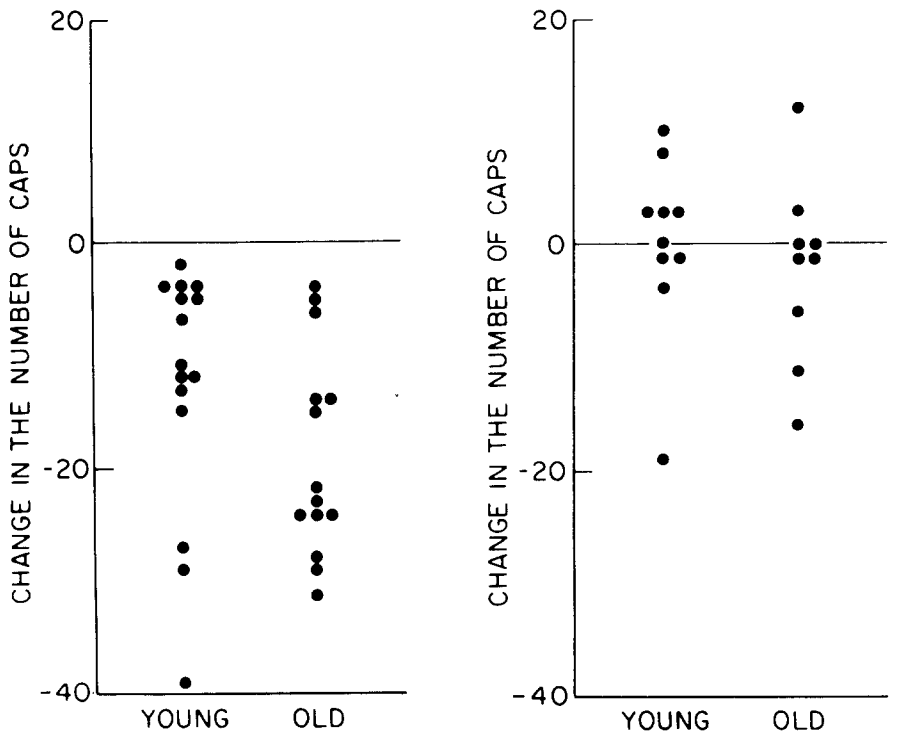

FIG. 4. Effect of colchicine plus cytochalasin B on con A-induced capping. Lymphocytes were preincubated with colchicine (10^{-5} *M*, final concentration) plus cytochalasin B (10^{-6} *M*, Fig. 4a; 10^{-7} *M*, Fig. 4b). Experimental protocol was similar to that described in Fig. 3.

fluence con A-induced capping (Bourguignon and Balazovich, 1980). Thus, the observations reported in this paper might be pointing to an important area governing cell function which might be relevant to the study of the aging process. Experimental approaches to the study of actin polymerization and calmodulin function in relation to age might yield fruitful results in our quest to understand the molecular mechanism of senescence.

SUMMARY

Con A-induced capping of lymphocytes is altered by a number of cytoskeletal modulating agents. Zinc, in the presence of colchicine, demonstrated an age-dependent differential effect on con A-induced capping of human peripheral blood lymphocytes. In general, zinc enhanced capping of lymphocytes from young individuals and suppressed from those of old individuals.

The effect of zinc on the cap formation adds further support to the evidence that zinc might be acting on the microfilaments. However, cytochalasin B, another microfilament modulating agent, does not show a differential effect similar to zinc. This suggests that zinc might modify microfilament formation or function in a manner different from cytochalasin B. The proposal that the effects of zinc might be due to its action on calmodulin suggests that calmodulin, the calcium regulating protein, may have a role in the physio-

logical processes associated with aging. Further studies in this area might yield fruitful results in our understanding of the molecular mechanism of senescence.

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