Clin. exp. Immunol. (1978) 32, 484–488.

Monocyte-dependent stimulation of human T cells by zinc

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(*Received* 12 *December* 1977

SUMMARY

Subpopulations of human peripheral blood leucocytes were isolated by nylon wool filtration or E-rosette separation and tested for functional activity. As shown previously, zinc ions induce DNA synthesis in unfractionated lymphocyte cultures. E-rosette-forming cells (E-RFC), obtained either by nylon wool filtration or E-rosette separation, responded well to PHA but showed only low levels of proliferative reactivity to zinc and Con A. These diminished responses could be completely restored by the addition of small numbers of autologous, mitomycin-treated monocytes; further experiments suggested that a monocyte-derived soluble factor can substitute for monocytes in this function. B lymphocyte-enriched cell populations, containing less than 1% E-RFC, did not respond to zinc and showed only marginal reactivity to PHA and Con A.

INTRODUCTION

The model of polyclonal lymphocyte activation has been used by cell biologists and immunologists to study the mechanisms by which resting lymphocytes are induced to proliferate and to divide. The mechanism of this activation is still unknown (for review see Ling, 1968; Oppenheim & Rosenstreich, 1976; Wedner & Parker, 1976). We have demonstrated that human peripheral lymphocytes can be stimulated by zinc ions (Kirchner & Rühl, 1970; Rühl, Kirchner & Bochert, 1971). Compared to the more commonly used phytomitogens, phytohaemagglutinin (PHA) and concanavalin A (Con A), zinc has several unique features: (a) its simple chemical structure can allow investigations not possible with phytomitogens; and (b) two clinical syndromes associated with zinc deficiency suggest that zinc might play a role in the immune response: the first is a hereditary immunodeficiency in calves with thymus aplasia (Brummerstedt *et al.*, 1971), and the second is a syndrome in man—acrodermatitis enteropathica—in which defects of cellular immunity are found (Michaelsson, 1974).

In the present report we describe further studies on the effect of zinc on human lymphocytes *in vitro*. Evidence will be presented that the response to zinc, as a T-cell mitogen, is critically dependent on the presence of monocytes.

MATERIALS AND METHODS

Preparation of lymphocytes. Heparinized venous blood was obtained from healthy donors and the mononuclear cells were isolated by Ficoll–Hypaque gradient centrifugation. 3–5 $\times$ 10^7 cells were transferred on to nylon wool columns prepared by the method of Aisenberg, Long & Wilkes (1974). After an incubation of 1 hr at 37°C the non-adherent cells were eluted and washed twice. About 40% of the initial cell number was removed from the columns. To separate cells which formed rosettes with sheep erythrocytes (E-RFC) from non-rosetting cells, sheep red blood cells (SRBC), pre-treated with neuraminidase, were mixed with 10^7 lymphocytes. The mixture was centrifuged (400 *g*) and incubated for 40 min. Interphase (population 'B') and pellet cells (population 'T') were collected and the latter were incubated for 5 min with a red cell lysis buffer to lyse the attached erythrocytes. All cell suspensions were washed three times.

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0099-9104/78/0600-0484$02.00

Preparation of monocyte-enriched cell populations. Ficoll–Hypaque-isolated mononuclear cells were incubated in plastic petri dishes for 90 min at 37°C. The non-adherent cells were removed by gentle shaking, the adherent cells were detached by vigorous shaking and incubated again in petri dishes for 60 min. The adherent cells recovered after the second incubation consisted of 70–90% monocytes as judged by morphological criteria and esterase activity These cells were pre-treated with mitomycin C (20 μg/ml, 30 min) and washed thoroughly.

Determination of T and B lymphocytes. The percentage of E-RFC was determined by the technique of Pang, Baguley & Wilson (1974). The percentage of cells with surface immunoglobulin (Ig) was determined by direct immunofluorescence using rabbit anti-human Ig sera (IgM, IgG and a polyvalent Ig; Behringwerke, Germany). By using these cell surface markers the following values were obtained (mean of ten experiments): (a) unseparated cells: 58% E-RFC, 14% Ig-positive cells and 17% monocytes; (b) nylon wool-filtered cells: 78% E-RFC, less than 2% Ig-positive cells and less than 1% monocytes; (c) population 'T': 94·5% E-RFC and less than 2% Ig-positive cells or monocytes; and (d) population 'B': 64% Ig-positive cells, less than 2% E-RFC and 34% monocytes.

Lymphocyte culture technique. Cells were suspended in RPMI 1640 medium supplemented with 10% heat-inactivated human AB serum, L-glutamine and antibiotics. 10^6 cells per ml were cultured in round-bottomed microplates (Greiner, Germany) at a volume of 0·2 ml/well. PHA, Con A and zinc chloride were added in optimal stimulatory doses, which were 1·0 μg/ml and 20·0 μg/ml and $2{\cdot}0 \times 10^{-4}$ mol/ml respectively. All cultures were done in quadruplicate; PHA-stimulated cultures were harvested after 72 hr and Con A- and zinc-stimulated cultures after 144 hr, which was found to be optimal in our system. The cultures were pulsed with [^{3}H]thymidine ([^{3}H]TdR, 1·0 μCi/well; sp. act. 2·0 Ci/mM; Radiochemical Centre, Amersham, England) for the final 18 hr and harvested with the aid of a semi-automatic sample harvester (Skatron, Norway) on filters, the radioactivity of which was counted in a liquid scintillation counter. The results are expressed as the mean counts per minute (ct/min) or as Δ ct/min (ct/min in stimulated cultures minus ct/min in unstimulated cultures). In some experiments monocyte-enriched cell suspensions (10^6 cells per ml) were cultured in 16 × 125 mm plastic tubes at a volume of 3·0 ml, with or without zinc. After 24 hr cell-free supernatants from these cultures were obtained and added to the nylon wool-filtered lymphocytes.

RESULTS

In the unfractionated lymphocyte suspensions high levels of [^{3}H]TdR incorporation in response to PHA, Con A and zinc were observed (Table 1). T cell-depleted lymphocyte suspensions (population 'B', containing predominantly B cells and variable numbers of monocytes) did not respond to zinc and showed only a marginal reactivity to PHA and Con A. Purified T cells, obtained either by nylon wool filtration or E-rosette separation, showed a decreased reactivity to PHA and Con A and virtually no response to zinc. The responses to PHA were less reduced than those to Con A.

TABLE 1. Reactivity of human lymphocyte subpopulations to mitogens. Subpopulations were obtained by nylon wool filtration or E-rosette separation; 2×10^5 lymphocytes in 0·2 ml complete culture medium were incubated with optimal doses of PHA, Con A and zinc chloride and cultured for 72 hr (PHA) or 144 hr (Con A and zinc). Results are expressed as the mean ct/min ± s.e. from twelve experiments (nylon wool filtration) and ten experiments (E-rosette separation)

Subpopulation	PHA	Con A	Zinc
Control	496±123	1406±387	
Unseparated	82259±10053	74421±8680	40880±2710
Control	612±86	1817±235	
Nylon wool-filtered	63610±4170	12184±1890	2906±374
Control	528±212	1687±168	
Unseparated	95278±8963	81703±5660	44371±9617
Control	886±112	1688±435	
'T' (E rosette-separated)	52496±9060	26403±3650	3398±183
Control	410±77	1086±118	
'B' (E rosette-separated)	3728±1040	5994±625	2268±453

TABLE 2. Monocyte requirement for mitogen-induced lymphocyte stimulation. Unseparated and nylon wool-filtered cells were incubated with optimal doses of PHA, Con A and zinc chloride respectively and cultured for 72 hr (PHA) or 144 hr (Con A and zinc). Autologous, mitomycin-treated monocytes were added to nylon wool-filtered cells at various concentrations. Results are expressed as the mean Δ ct/min ± s.e. from three experiments

Subpopulation	PHA	Con A	Zinc
Unseparated	88,510±7512	63,720±3518	44,075±5610
Nylon wool-filtered	51,718±8619	6016±512	1915±183
Plus 1% monocytes	103,715±6703	17,383±2781	16,006±985
Plus 2% monocytes	108,397±4898	29,917±1890	23,817±4917
Plus 5% monocytes	91,658±11605	58,309±4610	35,270±2609
Plus 10% monocytes	101,567±6585	53,687±7689	54,785±7250

TABLE 3. Effect of a monocyte-derived soluble factor on zinc-induced lymphocyte stimulation

Subpopulation	Experiment 1	Experiment 2	Experiment 3
Unseparated			
Control	1120±83*	740±103	1203±187
Zinc	28,499±2610	14,553±917	22,006±2714
Nylon wool-filtered			
Control	722±44	388±17	1067±140
Zinc	1718±63	742±29	2567±186
Control plus supernatant†	963±53	1632±127	1685±122
Zinc	8351±428	9467±615	9335±610
Control plus supernatant‡	1168±207	3213±283	2182±237
Zinc	14,527±2711	21,963±1713	16,124±917

* Results are expressed as the mean ct/min ± s.e. from quadruplicate cultures.

† 50% of a supernatant obtained from autologous monocytes cultured for 24 hr without zinc were added to nylon wool-filtered lymphocytes.

‡ 50% of a supernatant obtained from autologous monocytes cultured for 24 hr with zinc were added to nylon wool-filtered lymphocytes.

That the reduced mitogenic reactivity of T cell-enriched populations was caused by monocyte depletion was shown in the following experiments. In these, nylon wool-filtered lymphocytes were co-cultured with syngeneic, mitomycin-treated monocytes. As few as 1% monocytes caused a significant reconstitutive effect on the response to all mitogens (Table 2). Complete restoration of the reactivity to both Con A and zinc could be achieved with approximately 5–10% monocytes. To elucidate the action of monocytes, further experiments were done in which supernatants from monocyte-enriched cell suspensions were added to nylon wool-filtered lymphocytes. As shown in Table 3, the addition of 50% of these supernatants obtained from cultures after 24 hr of incubation increased the reactivity substantially.

DISCUSSION

Our data have shown that zinc induces DNA synthesis in cultures of human peripheral T lymphocytes while B cells do not respond. We cannot exclude, however, the possibility that in mixtures of T and B cells a small proportion of B lymphocytes can be stimulated by zinc, as shown for other mitogens which predominantly act on T cells (Greaves, Janossy & Doenhoff, 1974).

Furthermore, we have shown that activation of T lymphocytes by zinc requires the presence of monocytes; the same monocyte dependency has been shown for other stimulants. Thus it has been demonstrated that antigen-induced T-cell activation and the reactivity in the mixed leucocyte culture reaction are monocyte-dependent (for review see Oppenheim & Rosenstreich, 1976). There are also recent reports showing that the PHA and Con A reactivity of human (Hedfors, Holm & Petterson, 1975) and guinea-pig lymphocytes (Rosenstreich, Farrow & Dougherty, 1976) require the presence of monocytes or macrophages. The data of this study and a report showing that the stimulation of human peripheral lymphocytes by sodium periodate is monocyte-dependent (Biniaminov, Ramot & Novogrodsky, 1974) indicate that stimulation by simple chemical compounds is as equally monocyte-dependent as lectin-induced lymphocyte stimulation. Thus, at least in human lymphocyte cultures, T-cell activation by most stimulants appears to require the presence of monocytes. The number of these cells, however, necessary for optimal PHA stimulation and for the stimulation induced by Con A or zinc appears to be different. As previously shown (Rühl *et al.*, 1977), the reactivity to PHA is less sensitive to the removal of monocytes. This may indicate that, as in the data of Stobo, Rosenthal & Paul (1972) in mice, different T-cell subsets in the human peripheral blood are stimulated by different mitogens, the monocyte dependency of which varies. In this case, the zinc-responsive cell population appears to be more closely related to the Con A-reactive lymphocytes than to the PHA-reactive cells. It is nevertheless conceivable that the same subset of T cells responds to these stimulants. The stimulation of human peripheral lymphocytes by zinc and Con A has similar kinetics, in that the peak of reactivity is seen later than in PHA-stimulated cultures. One may assume that activation by mitogens which induce a delayed response is more dependent on the presence of monocytes than that of faster-acting stimulants, because of the tissue culture conditions.

Results from preliminary experiments shown here suggest that a monocyte-derived factor can replace the functions of these cells, at least to a certain extent. The existence of such factors is well established. Bach *et al.* (1970) were the first to report that the impaired responses of purified lymphocytes to allogeneic cells were restored by a supernatant factor obtained from cultured macrophages. The enhancing effect of such a factor on lymphocyte proliferation, which was termed 'lymphocyte activating factor' (LAF), was also shown for mitogen-induced T-cell proliferation (Gery, Gershon & Waksman, 1971, 1972; Gery & Waksman, 1972) and antigen-induced stimulation (Havemann & Schmidt, 1974). LAF has been also found to enhance the response of T lymphocytes to sodium periodate (Novogrodsky & Gery, 1972). These findings led to the assumption that T cells—similar to B lymphocytes—require two signals for activation (Oppenheim & Rosenstreich, 1976): the first signal is generated by the binding of activating substances and cross-linking of cell surface receptors while the second signal is delivered by monocytes or macrophages or by mediators derived from these cells.

The authors appreciate the technical assistance of Mrs Gudrun Bochert.

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