THE EFFECT OF ZINC CHLORIDE ON THE REDISTRIBUTION OF SURFACE IMMUNOGLOBULINS IN RAT B LYMPHOCYTES

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Received 11 September 1978
Revised version received 23 October 1978

1. Introduction

Several attempts have been made to substantiate the possible involvement of cytoskeleton components in cell surface receptor mobility [1–4]. Zinc could constitute a valuable tool for this purpose. Zinc is known to stabilize neurotubules in rat brain homogenate and to inhibit colchicine fixation [5]. Isolation of cell membranes in the presence of zinc is known to produce large fragments [6] containing cytoskeletal proteins [7]. In [8] the effect of zinc on the number and mobility of concanavalin A receptors in rat thymocytes at 4°C was investigated. We observed that zinc increased the number of surface receptors with a weak restriction of their mobility. Colchicine and cytochalasin B-sensitive structures were shown to modulate receptor mobility and density in this system [9,10]. In view of these findings, we decided to test the effect of zinc on the mobility of other surface receptors at 37°C, i e., B lymphocyte surface immunoglobulins. Peroxidase-labeled antibodies were used to follow the anti-immunoglobulin antibodies-induced redistribution of B lymphocyte surface immunoglobulins in the presence or absence of $ZnCl_2$.

2. Materials and methods

2.1. *Cells*

Mesenteric and cervical lymp nodes from month 5–7 male Fisher rats were teased in Hanks medium (Institut Pasteur) buffered with 10 mM Hepes (Sigma) pH 7.2 and containing 10% fetal calf serum (Gibco). The homogenate was passed through a nylon mesh to remove non-dissociated tissue and cells were washed twice in medium. The cell suspension was then adjusted to 5×10^7 cells/ml. Cell viability, as assessed by trypan blue dye exclusion, was ~95%

2.2 *Capping*

After incubation for 2 h at 37°C in the presence or absence of 50 μM zinc, the cells were incubated for a further 30 min at 4°C with 200 μg/ml peroxidase-labeled anti-rat immunoglobulin antibody (anti Ig-PO). The cells were then washed 3 times with medium and capping was allowed to proceed at 37°C. Reactions were stopped by placing the cells at 4°C after 10, 15 or 20 min. $ZnCl_2$ was present in the medium during the incubation with anti Ig-PO the subsequent washing and the capping reaction. Cells were cytocentrifuged, air-dried and fixed in 4% paraformaldehyde 0.2 M cacodylate buffer (pH 7.4) for 15 min at 20°C, washed 4 times in phosphate-buffered saline and the peroxidase activity was then revealed by the method in [11]. At least 1000 cells were examined for each experiment. Cells were scored as caps when the stain was detectable over no more than 1/3rd of their surface irrespective of whether it was continous or spotted. They were scored as patches when the stain was spotted and detectable over more than 1/3rd of the cell surface (fig.1). To compare these results we used statistical methods in [12].

2.3. *Material*

Anti Ig-PO were prepared as in [13]. $ZnCl_2$ was purchased from E. Merck A.G.

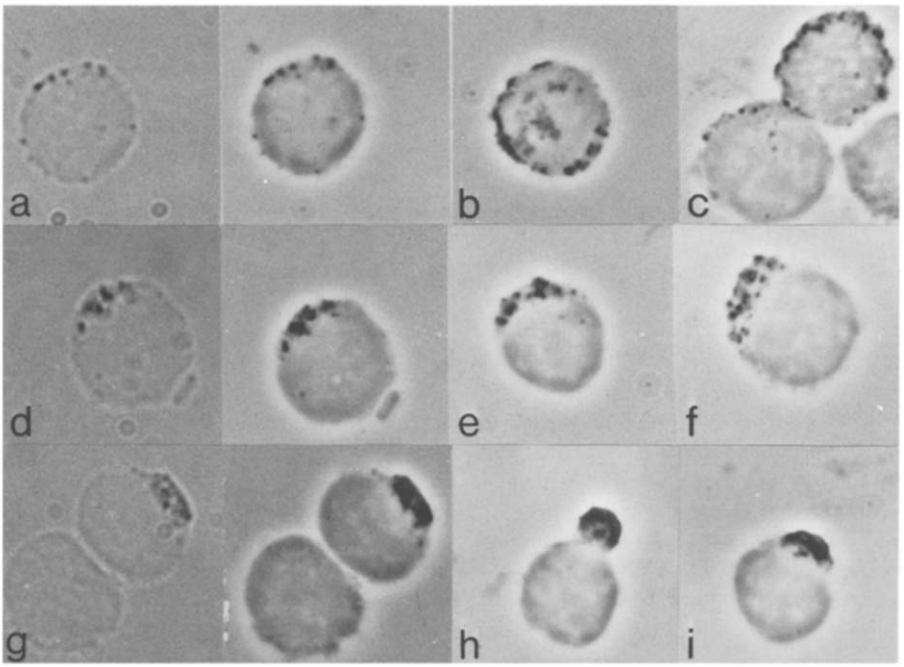

Fig.1 Distribution of anti Ig-PO on cells. (a–c) patches, (d–f) spotted caps, (g–i) homogeneous caps (a, d, g) comparison of the same peroxidase-labeled cells by light and phase contrast microscopy (b, c, e, f, h, i) phase contrast microscopy (× 1600).

3. Results

In 6 different control experiments 36.0 ± 2.1% of the cells were stained by anti Ig-PO. This corresponds to the % of B lymphocytes in lymph nodes. At none of the reaction times tested did we observe diffuse staining, but only caps and patches.

The effect of zinc at 50 μM was studied. This concentration was used in [8] on cell-surface lectin binding and lectin-induced blastogenesis and in addition was non-toxic for lymph node cells, as assessed by Trypan blue dye exclusion. In the presence of zinc 34 3 ± 3.1% of the cells were stained by anti Ig-PO. This is similar to the results observed in control experiments. Again we observed caps and patches only. However the number of cells which showed polarized staining was lower in the presence of zinc, whatever the reaction time. These results are shown in table 1. The inhibition due to $ZnCl_2$ was statistically significant for 10 min and 15 min in the capping reaction. A weaker, non-significant, effect was observed at 20 min.

Table 1
Effect of 50 μM zinc on anti Ig-PO-induced redistribution of B lymphocyte surface immunoglobulins

Time of reaction (min)	Caps %	
	Control	Zinc (50 μM)
10	39 9	31 1 $p < 0\ 02$
15	38.3	27 8 $p < 0\ 01$
20	40 8	36 0 $p \simeq 0\ 19$

4. Discussion

In this study the effect of zinc on the redistribution of B lymphocyte surface immunoglobulins induced by anti-Ig was investigated. An effect of zinc on the appearance of caps was observed. This effect

was maximum when zinc was added up to 15 min after the beginning of the capping reaction. After 20 min only a weak inhibition was observed. From these data we concluded that $ZnCl_2$ delayed the formation of caps. The dose of 50 μM zinc, which was used in these experiments, had no toxic effects and was 20-times lower than the dose used to prepare membrane fragments [2]. We therefore interpreted our results on capping as a true effect on cell-surface receptor mobility.

This result confirms the work in [14] on sheep red blood cell rosette formation by human lymphocytes in which 300 μM zinc inhibits the capping of these rosettes. It is also consistent with the results obtained on concanavalin A receptor mobility at 4°C in rat thymocytes [8]. In that case, a different experimental approach for measuring receptor mobility was used. However, in both systems, concanavalin A receptors on rat thymocytes at 4°C and surface immunoglobulins on rat B lymphocytes at 37°C, the same effect was observed. Zinc did not completely inhibit cell-surface mobility but only restricted it. Capping is likely to be dependent on cytoskeletal constituents as indicated by the effect of colchicine and cytochalasin B [1,3,4]. The delay of capping that we report here lends further support to an effect of zinc on membrane–cytoskeleton interactions.

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