PERTURBATION OF LEUKOCYTE METABOLISM BY NONPHAGOCYTOSABLE CONCANAVALIN A-COUPLED BEADS

D. ROMEO, M. JUG, G. ZABUCCHI and F. ROSSI
Departments of Biochemistry and General Pathology
University of Trieste, 34127 Trieste, Italy

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1. Introduction

Concanavalin A (ConA) has become an interesting probe for the study of the mechanism of metabolic stimulation of leukocytes concomitant with phagocytosis [1–4]. The binding of this lectin to polymorphonuclear leukocytes (PMNL) and macrophages leads to a perturbation of their oxidative metabolism, i.e. enhanced respiration and glucose oxidation, similar to that detectable when the challenge is performed with phagocytosable matter. Although we have gained much evidence that ConA is exerting its effect by promoting a rearrangement of the molecular organization of the phagocyte surface [1,3], the possibility of a contribution to the stimulation by pynocytosed lectin could not entirely be excluded. To rule out this possibility we have exposed leucocytes to ConA covalently coupled to nonphagocytosable beads. Andersson et al. [5] and Ono et al. [6] have shown that this lectin displays mitogenic activity also when bound to an insoluble carrier.

2. Methods

ConA (Sigma, USA) was conjugated with fluorescein thioisocyanate by the method of Rinderknecht [7]. The lectin was coupled to Sepharose 2B (Pharmacia, Sweden) essentially according to Porath et al. [8]. Twenty ml of bead suspension (average diameter 190 μm) were repeatedly washed and suspended in an equal volume of H_2O; after adjusting the pH to 11 with 0.1 N NaOH, cyanogen bromide, 0.5 g in 5 ml of H_2O, was slowly added at room temperature with stirring. After about 10 min at pH 11, the suspension was abundantly washed with cold water and resuspended in 20 ml of 0.1 M phosphate, pH 6.5. Both unlabelled and fluorescein tagged ConA, 120 mg in 20 ml of 2 M NaCl, were added, and the reaction mixture was gently stirred for 1 day at 0–4°C, before the beads were several fold washed on a filter with 0.154 M NaCl. The elution of unbound ConA in the washings was followed by protein determination [9] and by fluorometry. After standing for 1 day at 0–4°C, the suspension was filtered once more, and resuspended in fresh 0.154 M NaCl. Approximately 80–90% of the protein resulted to be covalently bound to Sepharose. Examination of the beads in a fluorescence microscope showed that they were undamaged and homogeneously labelled with fluorescein ConA. Isolation of guinea pig PMNL and rabbit alveolar macrophages (AM) and measurement of glucose oxidation were performed as previously described [10,11].

Correspondence to: Dr. D. Romeo, Istituto di Chimica Biologica, Università di Trieste, 34127 Trieste, Italia.

3. Results and discussion

Table 1 shows that PMNL and AM exposed to immobilized ConA become activated into increased 1-[^{14}C]glucose oxidation. As with soluble ConA the extent of activation is greater for PMNL than for AM (see insert of fig. 2 and refs 1,4]. Since the yield

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Table 1
Stimulation of glucose oxidation in phagocytes by immobilized concanavalin A

	1-[^{14}C]Glucose		6-[^{14}C]Glucose	
	Rest	+ ConA–Sepharose	Rest	+ ConA–Sepharose
PMN leukocytes	22 415	63 260	210	290
Alveolar macrophages	5125	7510	1525	1500

1×10^7 cells were incubated in 2.5 ml of calcium-free Krebs–Ringer phosphate (pH 7.4), containing 0.2 mM glucose, in the absence or in the presence of ConA covalently bound to Sepharose 2B (about 1.2×10^4 beads). The cell suspensions were subjected to gentle agitation in a metabolic shaker (75 oscillations/min). After 8–10 min at 37°C, 0.8 μCi of 1-[^{14}C]Glucose or 2 μCi of 6-[^{14}C]Glucose were added and the incubation continued for 10 min. Cell activity was stopped with 2 ml of 1 N H_2SO_4 and $^{14}CO_2$ trapped in KOH [11]. Data are expressed as $^{14}CO_2$ cpm/μCi of [^{14}C]glucose.

of $^{14}CO_2$ from 6-[^{14}C]glucose is not enhanced, the metabolic route stimulated appears to be the hexose monophosphate pathway. This result is consistent with previous conclusions reached with soluble ConA or phagocytosable matter [1,3,11]. Both the medium in which the ConA-binding beads were suspended and Sepharose activated with CNBr but not coupled to protein fail to exhibit any stimulatory effect. Cells do not adhere to CNBr derivatized Sepharose, whereas they get immediately bound to ConA-coupled beads (fig. 1).

By increasing the amount of immobilized ConA available to the cells, there is a linear increment of the extent of metabolic stimulation (fig. 2). The framed graph inserted in fig. 2 shows the stimulation of glucose oxidation by soluble ConA. In our view, a comparison of the stimulatory efficiency of immobilized ConA with that of soluble lectin is not feasable, since in the first case the number of cells having the possibility of interacting with the stimulant might be highly variable. In fact, this possibility depends on the number of ConA molecules present on the surface of the beads, on the frequency of collisions between cells and spheres, and probably on other factors which cooperate in anchoring the phagocytes to Sepharose upon the initial ConA-mediated binding.

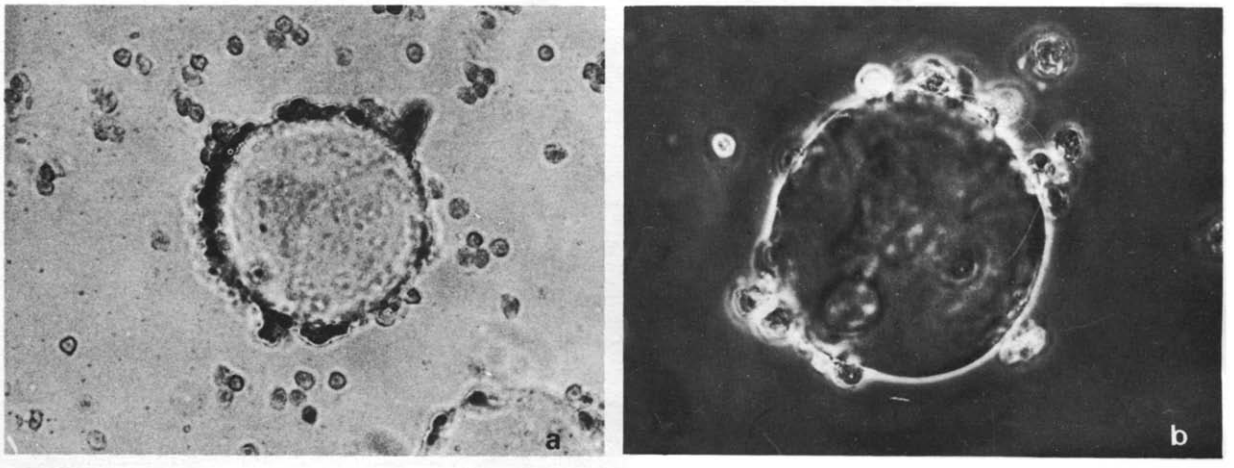

Fig. 1. Photographs of ConA-coupled Sepharose beads binding PMNL (A, 800 ×) or AM (B, phase contrast, 1250 ×).

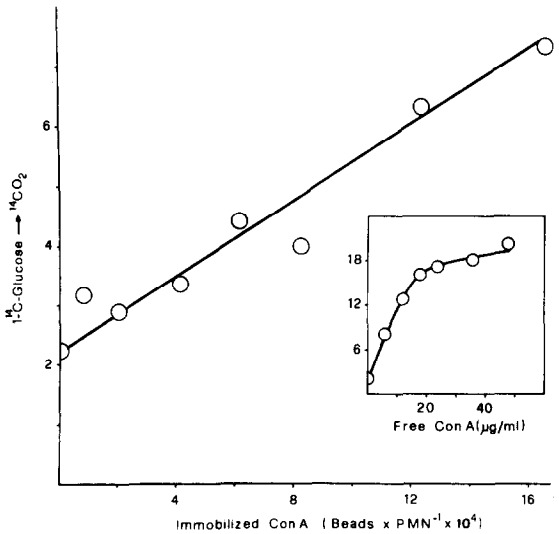

Fig. 2. Stimulation of glucose oxidation in PMNL by different amounts of ConA-coupled Sepharose beads or soluble ConA. Ordinates: cpm X 10^{-4}/μCi/10 min/1 X 10^7 cells. Note: Due to a more precise evaluation of the composition of the Concanavalin A preparation used, the concentrations of the lectin shown in a previous publication [1] should be multiplied by 0.125.

Pretreatment of ConA-Sepharose with a reagent known to have a high affinity for ConA, α-methyl-D-mannopyranoside [12], prevents both binding of cells and stimulation of their metabolism.

All these data strongly suggest that there is a correlation between cell adhesion to ConA-coupled beads and metabolic stimulation. Since the beads used are 6 to 25 times larger than the leukocytes, bound ConA is unavailable to the intracellular environment. Due to the short term incubations, it is unlikely that enzymes released from the phagocytes could remove a relevant amount of active lectin molecules from the beads.

Thus, the present findings confirm our previous suggestion that ConA transmits the information for stimulation of the oxidative metabolism of the phagocyte via an interaction with glycoprotein receptors localized on the cell surface and not in endocytic vesicles. Although the precise mechanism of metabolic perturbation by ConA is still to be elucidated, we have speculated that one of the primary events in this process might be a change in membrane ion permeability [1,2].

Hawkins [13] and Henson [14] have used immunocomplexes bound to a biosynthetic collagen polymer membrane or to micropore filters as surface stimulus. In their model systems, however, the proteins are not covalently linked to the nonphagocytosable matter and a careful scrutiny of their possible removal from the binding material is required. A strict similarity exists between our model and that of Melmon et al. [15], who have studied the binding of leukocytes to immobilized histamine.

All these experiments provide an interesting approach to the study of the relationship between metabolic stimulation of leukocytes and perturbation of their surface membrane by specific factors (enzymes, opsonis, hormones, immunoglobulins, etc.) independently of endocytosis. This approach should also offer a series of advantages in studying the process of immunological recognition by phagocyte receptors [16], by permitting a discrimination between recognition and ingestion.

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References

[1] Romeo, D., Zabucchi, G. and Rossi, F. (1973) Nature New Biol. 243, 111–112.
[2] Romeo, D. and Rossi, F. (1973) Acta Vitamin. Enzymol. 27, 145–150.
[3] Rossi, F., Zabucchi, G. and Romeo, D., in: Mononuclear Phagocytes in Immunity, Infection and Pathology (van Furth, R., ed.), in the press.
[4] Romeo, D., Zabucchi, G., Jug, M. and Rossi, F., Cell. Immunol., in the press.
[5] Andersson, J., Edelman, G. M., Möller, G. and Sjöberg, O. (1972) Eur. J. Immunol. 2, 233–235.
[6] Ono, M., Manita, H. and Mizuno, D. (1973) J. Biochem. (Tokyo) 73, 235–243.
[7] Rinderknecht, H. (1962) Nature 193, 167–168.
[8] Porath, J., Axén, R. and Ernback, S. (1967) Nature 215, 1491–1492.
[9] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[10] Rossi, F. and Zatti, M. (1964) Brit. J. Exp. Pathol. 45, 548–559.

[11] Romeo, D., Zabucchi, G., Marzi, T. and Rossi, F. (1973) Exp. Cell Res. 78, 423–432.
[12] Goldstein, I. J., Hollerman, C. E. and Smith, E. E. (1965) Biochemistry 4, 876–883.
[13] Hawkins, D. (1971) J. Immunol. 107, 344–352.
[14] Henson, P. M. (1971) J. Immunol. 107, 1547–1557.
[15] Melmon, K. L., Bowine, H. R., Weinstein, J. and Sela, M. (1972) Science 177, 707–709.
[16] Rabinovitch, M. and De Stefano, M. J. (1973) J. Immunol. 110, 695–701.