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EFFECT OF CONCANAVALIN A AND ITS SUCCINYLATED DERIVATIVE ON THE OXIDATIVE METABOLISM OF GUINEA PIG PERITONEAL MACROPHAGES

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Summary

The effect of concanavalin A and its succinylated derivative on the metabolic regulation of guinea pig peritoneal macrophages was observed. The binding of tetravalent concanavalin A to the surface glycoproteins of macrophages caused a marked increase in the rate of oxygen consumption due to the activation of the hexose monophosphate shunt. Divalent succinylated concanavalin A, also induced a similar change in the rate of oxygen metabolism. The metabolic change induced by these two types of lectin was reversibly inhibited by α-methyl-D-glucoside, a haptenic inhibitor of these lectins, and was temperature dependent (observed at above 15°C). It is suggested that the binding of these lectins to the surface glycoproteins, and not their cross-linking into caps, is required for the activation of oxygen metabolism of macrophages, and that highly fluid state of the plasma membrane seems to be an essential requirement for the transduction of glycoprotein perturbation on the macrophage surface into cellular interior via transmembrane control mechanism.

Introduction

The adhesion of substances to macrophage cell surface is a prerequisite for the triggering of phagocytosis [1,2]. The process of phagocytosis in macrophages as well as in polymorphonuclear leukocytes is accompanied by various kinds of metabolic change in these cells, such as increased respiration [3,4], enhanced glucose oxidation through the hexose monophosphate shunt [5,6] and increased synthesis of highly-reactive products such as H_2O_2 and O_2^- [7,8].

Similar changes in leukocyte metabolism can also be induced by various reagents such as phospholipase C [9], antileukocyte antibody [10], cytochalasin E [11] and concanavalin A [12] without causing phagocytosis. Among these metabolic inducers, concanavalin A selectivity interacts with mannose-

like sites of the cell surface [13,14]. Because of its specific binding activity, concanavalin A has become an useful probe for the study of membrane structure [15—17], cell recognition [18] and cellular metabolic regulation [5,19, 20].

Romeo et al. [5] reported that surface perturbation of leukocytes by concanavalin A induced stimulation of the oxidative metabolism. Tetravalent concanavalin A is known to induce surface glycoprotein cross-linking into micropatches and, eventually, caps [21]. Such a kind of cross-linking of glycoproteins on cell surface may contribute to transmembranous control mechanism [22]. The divalent succinylated derivative of concanavalin A is believed to have no such cross-linking activity of the receptors, and can induce neither capping of lymphocyte surface receptors nor agglutination of tumor cells. However, this derivative is known to induce certain biological changes in these cells such as blastoid transformation of lymphocytes and inhibition of tumor cell growth.

Similar dissociating phenomena between the two types of the lectins were observed with mouse peritoneal macrophages [23]. Tetravalent concanavalin A was reported to induce surface receptor cross-linking followed by a marked vacuole formation of the treated cells [24—27], while divalent derivative had no such activities.

The reason why some biological process induced by the lectins require high valence (tetravalency) while other processes do not, remained obscure. To understand transmembranous control mechanism, it seems to be necessary to study what kind of biological processes require receptors cross-linking, and what kind of processes do not.

In this communication, we observe the effect of valency of concanavalin A and the incubation temperature on the metabolic change in guinea pig peritoneal macrophages as well as polymorphonuclear leukocytes.

Materials and Methods

Materials

Concanavalin A was purified from Jack bean meals by the method of Agrawal and Goldstein [28]. Succinylated derivative of concanavalin A was prepared by the method of Gunther et al. [21], and further purified by affinity chromatography of Sephadex G-100. The two types of lectin thus obtained were dissolved in Hanks balanced saline solution, and used for the experiment. α-Methyl-D-glucoside was obtained from Sigma Chemical Co. (U.S.A.). All reagents were of analytical grade.

Cells

Peritoneal macrophages were collected from peritoneal exudates of Hartley guinea pigs (400—500 g body weight). After four days of a single intraperitoneal injection of liquid paraffin (20 ml), or 0.2% oyster glycogen dissolved in sterilized phosphate buffered saline (30 ml), the animals were bled to death and the cells were obtained from peritoneal cavity. The cells were washed three times with ice-cold Hanks balanced saline solution. The purity of peritoneal macrophages thus obtained was over 80%, and contaminant polymorphonuclear leukocytes were below 1.0% on the Giemsa staining method. Polymorpho-

nuclear leukocytes were collected from peritoneal exudates of guinea pigs after 4 h of a intraperitoneal injection of 0.2% oyster glycogen solution. Preparation of the cells was carried out as in the macrophages. The purity of polymorphonuclear leukocytes was higher than 94%, and no significant amount of contaminant cells such as lymphocytes or macrophages was observed in the cell preparation used for this experiment as checked by Giemsa stain. Viability of the cells was determined by the trypan blue dye exclusion test. No cell disruption was observed under the conditions used in the experiment.

Measurement of oxygen metabolism

The rate of oxygen consumption of peritoneal exudate cells was measured by a Clark type oxygen electrode in a 3-ml closed glass chamber. Krebs-Ringer phosphate buffer (pH 7.4) containing 0.2 mM D-glucose was used for the reaction mixture. The rate of oxygen consumption was recorded continuously at various temperatures. During the course of incubation of peritoneal cells ($2-6 \cdot 10^6$/ml), varying concentrations of the lectins were added to the incubation mixture. The extent of enhanced oxygen consumption induced by lectins was expressed as the proportion of the rate of oxygen consumption before and after adding lectins as follows:

$$\text{Activation ratio} = \frac{\text{rate of } O_2 \text{ consumption after adding lectins } [O_2, \mu M/\text{min}]}{\text{rate of } O_2 \text{ consumption before adding lectins } [O_2, \mu M/\text{min}]}$$

Results

Changes in the rate of oxygen consumption of polymorphonuclear leukocytes

Fig. 1 shows the effect of tetravalent concanavalin A and its divalent succinylated derivative on the rate of oxygen consumption of polymorphonuclear

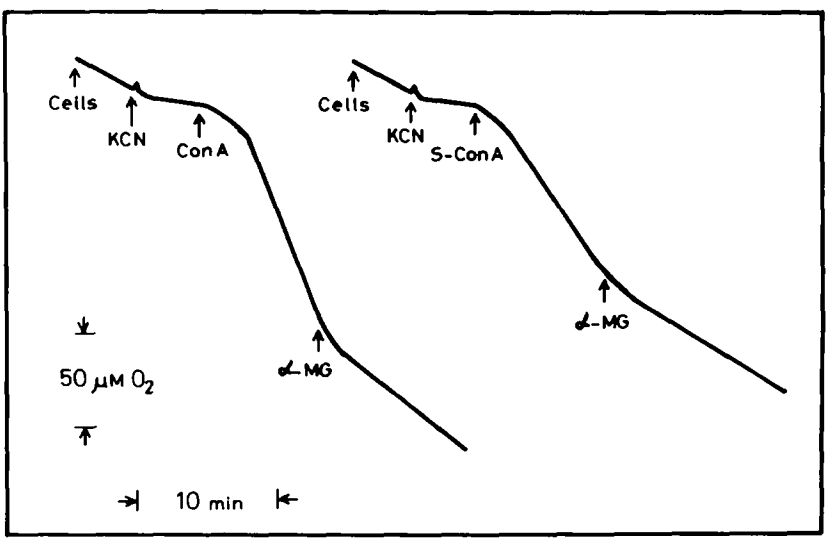

Fig. 1. Effect of tetravalent concanavalin A (Con A) and divalent succinylated concanavalin A (S-Con A) on the oxygen consumption of polymorphonuclear leukocytes. Polymorphonuclear leukocytes ($4.4 \cdot 10^6$/ml) were incubated at 37°C and either 100 μg/ml tetravalent concanavalin A or divalent succinylated concanavalin A was added to a 3-ml glass chamber. Concentrations: α-methyl-D-glucoside (α-MG), 10 mM; KCN, 1 mM.

leukocytes. Addition of native concanavalin A to the incubation mixture caused a rather rapid and marked increase in the rate of oxygen consumption of polymorphonuclear leukocytes, showing good agreement with the data reported by Romeo et al. [5].

Similar change in the metabolic activation of polymorphonuclear leukocytes was also induced by divalent succinylated concanavalin A. Both of the activated oxygen consumption by the two types of lectins were reversibly inhibited by α-methyl-D-glucoside.

Changes in the rate of oxygen consumption of macrophages

Fig. 2 shows the effect of two types of lectin on the rate of oxygen consumption of peritoneal macrophages. Similar metabolic change of peritoneal macrophages was induced by either tetravalent concanavalin A or succinylated concanavalin A, and was reversibly inhibited by α-methyl-D-glucoside, as was the case for polymorphonuclear leukocytes. The activation ratio of oxygen consumption induced by these lectins, however was significantly lower in macrophages than in polymorphonuclear leukocytes.

Dose-dependent activation ratio of oxygen consumption of macrophages

Fig. 3 shows the effect of two types of the lectin over a range of concentration from 5 to 100 μg/ml on the metabolic activation ratio of peritoneal macrophages. The induction of enhanced oxygen consumption followed saturation kinetics as a function of lectin concentration. A maximal activation ratio of oxygen consumption was obtained at the lectin concentration of 100 μg/ml with both types of lectin, and no further increase in the activation ratio was observed at higher concentration of the lectins.

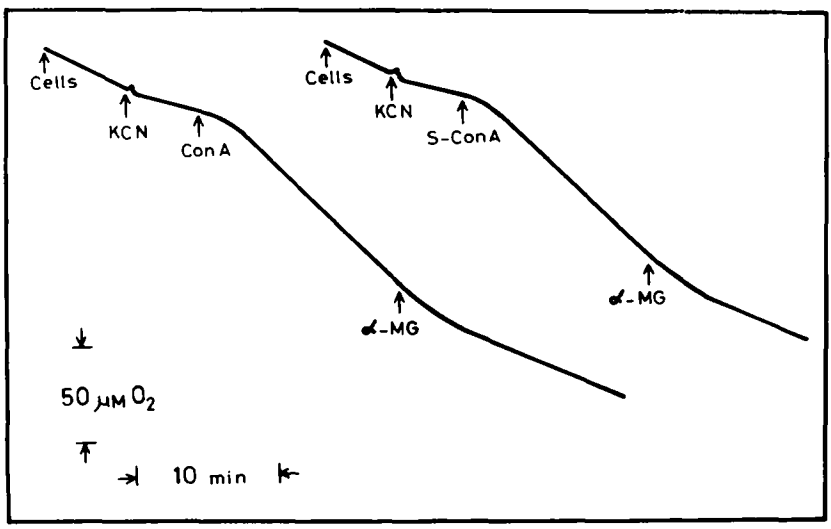

Fig. 2. Effect of tetravalent concanavalin A (Con A) and divalent succinylated concanavalin A (S-Con A) on the oxygen consumption of peritoneal macrophages. Macrophages ($5.5 \cdot 10^6$/ml) were incubated at 37°C and either 100 μg/ml tetravalent concanavalin A or divalent succinylated concanavalin A was added to a 3-ml glass chamber. α-Methyl-D-glucoside (α-MG), 10 mM; KCN, 1 mM.

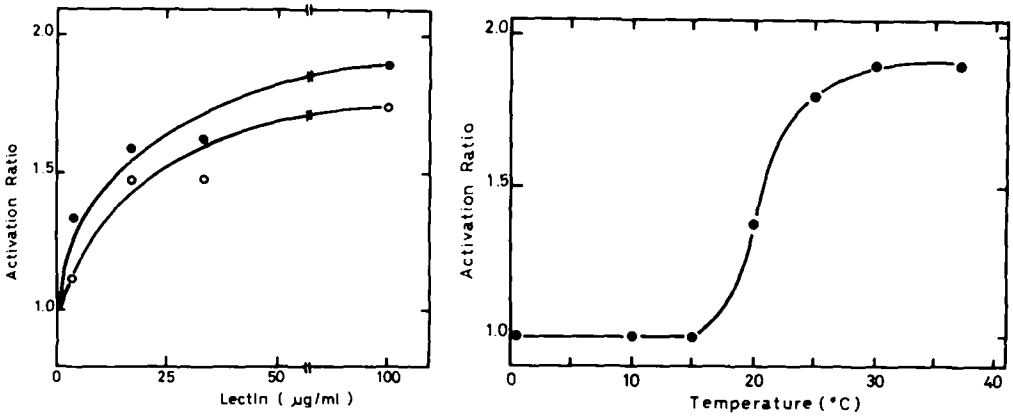

Fig. 3. Effect of doses of the two types of concanavalin A on the activation ratio of oxygen consumption. Peritoneal macrophages ($4 \cdot 10^6$/ml) were incubated and various doses of tetravalent concanavalin A (●——●) or divalent succinylated concavanalin A (○——○) were added.

Fig. 4. Effect of temperature on the activation ratio of oxygen consumption induced by tetravalent concanavalin A (100 μg/ml). Peritoneal macrophages used were $4 \cdot 10^6$/ml. Other conditions were the same as in Fig. 1.

The activation ratio induced by tetravalent concanavalin A was somewhat higher than that induced by succinylated concanavalin A at any concentration of the lectins observed.

Temperature-dependent response of macrophages

The effect of temperature on the metabolic activation ratio of macrophages is shown in Fig. 4. The ratio of metabolic activation of macrophages was temperature dependent, and was observed at higher temperature above 20°C. Under 15°C, no significant enhancement of the rate of oxygen consumption observed.

Discussion

The data show that the binding of concanavalin A or its succinylated derivative to the surface membrane of guinea pig peritoneal macrophages caused a marked enhancement of the rate of oxygen consumption. The phenomena observed with two types of the lectin were inhibited reversibly by α-methyl-D-glucoside, a well-known haptenic inhibitor of the lectins. These data showed a good agreement with the data of polymorphonuclear leukocytes using tetravalent concanavalin A reported by Romeo et al. [5]. The extent of the metabolic activation depended on the lectin concentration and incubation temperature. Marked activation of the rate of oxygen consumption was observed only above 15°C.

Concanavalin A and its succinylated derivative could induce metabolic activation of either polymorphonuclear leukocytes or macrophages, however the extent of enhanced rate of oxygen consumption of the former was significantly high compared with that of the latter. The observed change in oxygen metabolism of both types of the cells reflected the activation of CN^--insensitive

hexose monophosphate shunt as suggested by Romeo et al. [5]. The exact reason for this different effect of the lectins on the two types of cell remained unclear. Polymorphonuclear leukocytes, having a rather small amount of mitochondria, depend mainly on glycolysis and the hexose monophosphate shunt pathway for their energy metabolism, while peritoneal macrophages, having a sufficient amount of mitochondria, depend on glycolysis as well as oxidative phospholylation of mitochondria [29]. This seems likely to account for the different effect of the lectins on the extent of metabolic activation of the two types of cell. Though the ratio of metabolic activation of macrophages induced by tetravalent concanavalin A was somewhat higher than that induced by divalent succinylated concanavalin A, both types of the lectin could induce the enhanced oxygen consumption, indicating that the valency of the lectin is by no means an essential factor for the metabolic activation.

In contrast, Goldman et al. [23] reported that tetravalent concanavalin A, but not the divalent one, induced a marked vacuole formation of mouse peritoneal macrophages within 30 min. The inducing activity of the lectins was reported to be significantly enhanced by the pretreatment of the cells with colchicine, a well known inhibitor of cytoskeletal systems [27]. They suggested that, for the induction of such a morphological change, the polyvalency of lectins and their molecular size were important factors which might cause surface receptor cross-linking, and that some cytoskeletal systems were involved in the regulation of sequential events leading to lectin-induced vacuolation.

Contrary to this, metabolic activation of macrophages observed in the present report was found to be independent on the valency and was induced in less than 1 min. Preliminary experiments showed that the lectin-induced metabolic burst of macrophages was not inhibited by the pretreatment of the cells with colchicine (data not shown). Thus, it seems likely that the vacuole formation and the induction of metabolic activation of the cells by the lectin are processes independent from each other.

From these data it was suggested that only binding of concanavalin A or its derivative to the mannose-like glycoproteins might be required for the transmembranous information transfer system linked to the oxygen metabolism, or hexose monophosphate shunt. Of course, tetravalent concanavalin A caused a marked agglutination of macrophages, while the divalent derivative had no such activity (data not shown). Furthermore, this derivative was reported to have no activity of inducing patch-cap formation of macrophages [23], as was the case in lymphocytes [21]. However, it remains possible that, like divalent antibodies, succinylated concanavalin A can induce a very low amount of microaggregation of the receptors which may be sufficient for the metabolic activation of macrophages. Such a possibility may be defined by using monovalent concanavalin A derivative.

The activation of the rate of oxygen metabolism of leukocytes critically depends on the incubation temperature, and is observed above 15°C. Biological membrane is believed to be in a highly fluid state which may enable lateral movement of integral proteins as well as their conformational change. The latter may be involved in the transmembranous information transfer mechanism of metabolic burst of leukocytes observed in the present report.

Inoue et al. [30,31] reported that the tetravalent concanavalin A, but not

divalent succinylated concanavalin A, caused a marked induction of potassium release from the cells such as Ehrlich ascites tumor cells or rabbit erythrocytes. However, no such a change in K^+ compartmentation across plasma membrane was detected in the case of leukocytes incubated with tetravalent concanavalin A or divalent succinylated concanavalin A [32]. This indicates that the change in the potassium compartmentation across the plasma membrane is unlikely to account for the essential factor of transmembrane control mechanism of leukocyte oxygen metabolism. In this regard, based on the experiment using various ionophores such as valinomycine, X537A, and A23187, Zabucchi et al. [33] suggested that the activation of oxygen uptake by leukocytes was caused by an intracellular redistribution of cations, which might involve a Mg^{2+}-dependent mechanism. Whether such a change in ion compartmentation is triggering the concanavalin A-induced enhancement of oxygen consumption or not remains unclear, and now is under investigation by us.

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