Stimulation of the Respiration of Polymorphonuclear Leucocytes by Antileucocyte Antibodies

Phagocytosis or treatment with surface active agents induce in polymorphonuclear (PMN) leucocytes a dramatic increase of the oxygen uptake together with an enormous increase of glucose oxidation through the monophosphate shunt[1-8]. The fact that the metabolic stimulation occurs within a few seconds[5-8] and is elicited only when intact cells are used[9], suggests that the contact between the cell surface and the activating agents represents the trigger mechanism of the metabolic events. Besides the problems of the oxidative enzymes involved in the stimulation of the respiration, which is still controversial[2,7,10-14], and of the functional significance of the metabolic modifications, the key problem remains a better definition of the structural changes of the surface membrane which seem to be relevant in the regulation of the metabolic behaviour of PMN leucocytes.

The effect of anticellular antibodies alone or with complement (C′) on structural, biochemical and functional properties of many cells has been extensively investigated[15-17]. The present communication deals with the changes of the oxidative metabolism of PMN leucocytes exposed to specific antibodies.

The methods for the collection of leucocytes from guinea-pig exudates, for incubation and for biochemical assays, have been previously described[7,8,10]. Antiserum was prepared in adult rabbits by injecting i.v. $7-10 \times 10^7$ (7–10 mg protein) PMN leucocytes, freed of contaminating red cells by hypotonic hemolysis, washed and suspended in Krebs-Ringer phosphate. Seven such injections were made at 7-day intervals. 5–10 days after the last injection blood was collected from treated and control rabbits and serum separated. Inactivation of complement was achieved by heating for 30 min at 56 °C. Gamma-globulins were prepared by ammonium sulphate precipitation and purification on Sephadex G-200.

The results reported in the Figure show that immune serum causes in PMN leucocytes a great stimulation of the respiration within a few seconds. This stimulated respiration is unaffected by rotenone, antimycin A and cyanide and is associated to increased activity of the hexose monophosphate pathway as shown by the $C^{14}O_2$ production from glucose-1-C^{14} (Table). When purified immune gamma-globulins were used, similar effects on the respiration and $C^{14}O_2$ production were obtained. The modifications of the oxidative metabolism are similar to those induced by phagocytosis and by surfactants.

It is worthwhile pointing out that the effects we have shown are independent of complement. The metabolic stimulation of PMN leucocytes can be explained as the effect of the reaction of the specific antibodies at the cell surface. The binding of antibodies to the antigenic determinants of the plasma membrane causes molecular changes or rearrangements which could switch on the activation of the enzymes of cytosol or of granules. It has been shown that anticellular antibodies, in absence of complement, acting solely at the surface of the cells, can cause changes also of the cytoplasmic structures[18,19]. If this interpretation is correct, a better definition of the molecular sites involved in the reaction antigen-antibody could be a useful approach for understanding the relationship between the plasma membrane function and the metabolic activity of PMN leucocytes. The possibility that the metabolic modifications induced by antileucocyte antibodies are partially related to the direct effect against antigens of cytosol or of granule membrane can-

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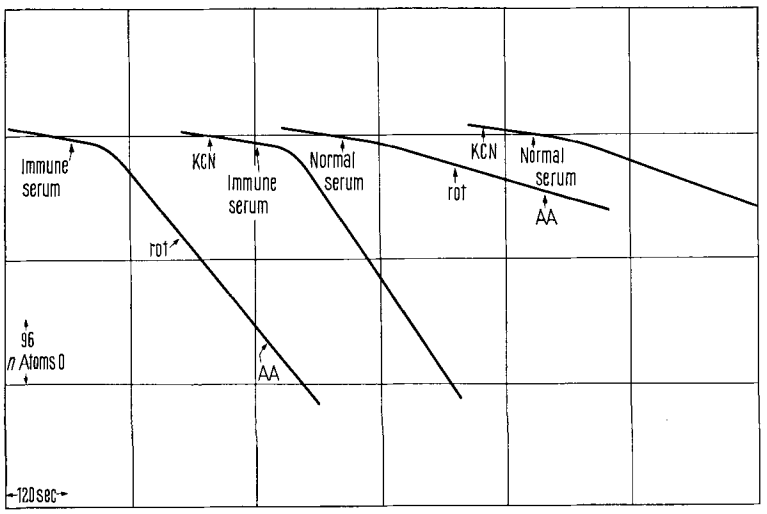

Effect of immune serum on the respiration of polymorphonuclear leucocytes. Recording of the oxygen concentration by Clark electrode. The incubation medium contained: 2×10^7 cells in 1.9 ml of calciumfree Krebs-Ringer phosphate containing 2.8 m*M* glucose. Rotenone (Rot) 5 μmoles. Antimycin (AA) 2 μg. KCN 4 μmoles. Serum 0.1 ml. Temperature 37 °C.

Effect of immune serum on $C^{14}O_2$ production from glucose-1-C^{14} by polymorphonuclear leucocytes (PMNL). Each vessel contained 2×10^7 cells in calcium-free Krebs-Ringer phosphate and 1 μc of glucose-1-C^{14} in 5.55 μmoles of 1-C^{14} glucose (radioactivity 8.5×10^4 counts/min/1 μmole); 0.1 ml serum or 10.5 mg γ-globulins when indicated. The values are given for 2×10^7 cells and for 20 min of incubation.[a]

	PMNL control	PMNL + normal serum	PMNL + immune serum	PMNL + normal γ-globulins	PMNL + immune γ-globulins
$C^{14}O_2$ from glucose-1-C^{14} (counts/min)	1,690	2,370	15,529	1,740	8,150
Glucose equivalents (nmoles)	19.9	27.9	182.6	20.5	95.8

[a] The experiment reported in this table is a typical one and has been selected from nearly 20 experiments. 6 different immune sera, each of them at several stages of immunization, were tested. The stimulation of $^{14}CO_2$ production induced by immune serum relative to that induced by normal serum ranged from 4 to 12 times, using different sera under the experimental conditions indicated in this table.

not be ruled out. However, it has been found that antiserum to leucocyte granule membranes has a stabilizing action on isolated granules[20].

It is known that leucocytes are able to pinocytose macromolecular substances, including proteins[21,22]. The effect of pinocytosis on the activity of some dehydrogenase in guinea-pig polymorphonuclear leucocytes has been reported[22]. On this line it is possible that the specific binding of immune gamma-globuline to antigenic determinants of the plasma membrane of PMN leucocytes facilitates the pinocytosis uptake of the globuline. In this case, it remains to be ascertained whether the plasma membrane modifications induced by the specific binding of antibodies represent per se the trigger mechanism for the stimulation of respiration of PMN leucocytes or whether an increased pinocytosis must occur as an intermediate step.

Studies are now in progress on the effect of antileucocyte antibodies on the glycolytic activity, on the oxidative enzymes involved in the stimulated oxygen uptake, and on the morphological modifications of the plasma membrane and of the granules[23].

Riassunto. Gli anticorpi antileucociti provocano una marcata stimolazione della respirazione, rotenone, Antimicina A e cianuro insensibile, ed una aumentata attività del ciclo degli esosomonofosfati nei leucociti di cavia.

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The Metabolism of Tri-Alkyl Phosphates[1]

Tri-alkyl phosphates (1) are reactive towards nucleophiles and have been used to alkylate a variety of functional groups[2]. The di-alkyl phosphate entity (usually dimethyl or diethyl) forms an integral part of a number of organophosphorus insecticides and recently trimethylphosphate (TMP, I $R = CH_3$) has been shown to possess a 'functional' sterilizing action in male rodents[3]. The metabolism of organophosphorus insecticides and tri-aryl phosphates is well documented but little is known about the fate of the simple tri-alkyl phosphates, even though they are known to react chemically by an alkylating mechanism.

Chromatograms of rat and mouse urine from either oral or intraperitoneal administration of ^{32}P-TMP (100 mg/kg and 1 g/kg respectively) revealed one radioactive area corresponding to ^{32}P-dimethylphosphate (II, $R = CH_3$). At similar dose levels ^{32}P-triethylphosphate (TEP, I $R = CH_2CH_3$) was excreted by both species as ^{32}P-diethylphosphate (II, $R = CH_2CH_3$). Apart from traces of trimethylphosphate in rat urine within 6 h of i.p. administration, in neither case was the tri-alkyl phosphate excreted unchanged and mouse bladders cannulated 1 h after oral dosing (1 g/kg) contained only the corresponding ^{32}P-metabolite. ^{32}P-TMP and ^{32}P-TEP are rapidly excreted as their metabolites by both rat and mouse with 90% recovery of radioactive material in urine within 16 h and nearly complete recovery over 96 h.

Both ^{32}P-dimethylphosphate[4] and ^{32}P-diethylphosphate are excreted unchanged, there being no mono-alkyl phosphate or phosphoric acid produced in either case. This contrasts with previous work in which di-alkyl phosphates[5], particularly dimethylphosphate[6,7] and diethylphosphate[8], have been reported to be metabolized to the mono-alkyl phosphate and phosphoric acid. However, as these products were obtained from dialkyl-aryl phosphates, they would be produced by de-arylation of initially de-alkylated metabolites. The organophosphorus compounds[9] *trans*-phosdrin[10], *cis*- and *trans*-bomyl[10] and troline[4], for example, are metabolized by de-arylation solely to dimethylphosphate whereas tri-aryl phosphates, such as tri-*o*-cresyl phosphate[11], are degraded through their di- and mono-aryl derivatives to phosphoric acid. The biological mono dealkylation of TMP and TEP, therefore, appears analogous to their chemical reactivity in which further de-alkylation occurs only under extremely severe conditions[12,13].

With uniformly-labelled ^{14}C-TMP, 2 further urinary metabolites were detected and identified as *S*-methyl cysteine (III, $R = CH_3$) and its *N*-acetate indicating that TMP, at least as far as its detoxification is concerned, acts in an alkylating capacity. Similarly triethyl-, tri-*n*-propyl, tri-*iso*-propyl- and tri-*n*-butyl-phosphates, apart from being excreted as the di-alkyl phosphate (II), gave rise to the corresponding *S*-alkyl cysteine (III) so that