STIMULATION OF GLUCOSE OXIDATION IN HUMAN POLYMORPHONUCLEAR LEUCOCYTES BY C3-SEPHAROSE AND SOLUBLE $C\overline{567}$

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1. Introduction

The process of phagocytosis in polymorphonuclear leucocytes (PMN) is associated with a number of metabolic modifications of leucocytes such as increased respiration, increased glucose oxidation through the hexose monophosphate shunt (HMS) and increased production of highly reactive molecules (H_2O_2 and O_2^-) [1–4]. A metabolic burst similar to that caused by phagocytosable materials can also be induced, in vitro, by producing a perturbation at the surface membrane of the cell with phospholipase C [5], antileucocyte antibodies [6], endotoxins [7], Concanavalin A [8], detergents [9] or fatty acids [10].

A stimulation of leucocyte functions by non-phagocytosable materials may also occur in vivo. It has been shown in fact that activation of the complement through the alternate pathway causes a stimulation of PMN metabolism [11] and induces selective exocytosis of granule enzymes in these cells [12]. The biologically active fragment C5a is considered to be responsible for both events [13,14].

In this paper we show that the interaction between C3b of $C\overline{567}$ and the cell surface of PMN plays a role in activating the oxidative metabolism of these cells.

2. Materials and methods

2.1. *Separation of PMN*

Leucocytes were separated from fresh human blood collected in ACD. 1 ml of 15% dextran solution (mol. wt 200 000) in isotonic saline was added to 6 ml of blood. After sedimentation of erythrocytes, leucocytes were harvested by centrifugation and residual erythrocytes lysed by brief exposure to hypotonic treatment. The differential counts gave on average the following percentages: PMN 80–90%, lymphocytes 10–20%, eosinophils 3–6%. Leucocytes were suspended in Hank's balanced salt solution (HBSS).

2.2. *Determination of glucose oxidation*

The production of $^{14}CO_2$ from 1-[^{14}C]- and 6-[^{14}C]-glucose was measured as previously reported [15].

2.3. *Preparation of C3-Sepharose*

C3-Sepharose was prepared according to Pepys et al. (personal communication, to be published). Briefly, packed Sepharose 4B (Pharmacia, Sweden), after washing with HBSS, was mixed with fresh human serum (ratio 1:10 v/v) and incubated at 37°C for 1 hr with constant stirring. Control Sepharose was prepared by incubating the beads with serum in EDTA (0.01 M). C3-Sepharose and control Sepharose were extensively washed after incubation. The presence of C3 bound to Sepharose was documented with fluorescein labelled anti human C3 (Behringwerke, Germany).

2.4. *Preparation of Con A-Sepharose*

Concanavalin A (Con A), obtained from Sigma Chemical Co., USA, was coupled to Sepharose 4B following the method described by Romeo et al. [8]. Trace amount of fluorescein-Con A was mixed with the unlabelled Con A during the process of Con A-Sepharose preparation to ascertain the binding of Con A to the beads.

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Preparation of $C\overline{567}$, $C\overline{56}$ and C7 were separated from human serum according to Lachmann and Thompson and Thompson and Lachmann, respectively [16–17].

3. Results

The binding of C3 to Sepharose beads was detected at the fluorescence microscope after treatment of the beads with fluorescein labelled anti C3 serum. The fluorescence was homogeneously diffused on the surface of the beads. A similar pattern of labelling was detected in Sepharose beads treated with fluoresceinated Con A. Table 1 shows the results of [^{14}C]-glucose oxidation by PMN in presence of control Sepharose and C3-Sepharose (ratio beads: leucocytes, 1:450). A five fold increase of $^{14}CO_2$ production from 1-[^{14}C]glucose took place when leucocytes were challenged with C3-Sepharose, whereas the production of $^{14}CO_2$ from 6-[^{14}C]glucose was unchanged indicating that the increase of glucose oxidation was performed by an enhanced activity of the hexose monophosphate shunt. The challenge of PMN with control Sepharose did not modify the rate of the oxidation of either 1-[^{14}C]glucose or 6-[^{14}C]glucose. In parallel experiments Sepharose with Con A immobilized on the surface induced also an increase of $^{14}CO_2$ production from 1-[^{14}C]-glucose as previously described in our laboratory [8].

Table 1
Effect of bound C3b on the stimulation of glucose oxidation in human polymorphonuclear leucocytes

	counts/15 min	
	1-[^{14}C]glucose	6-[^{14}C]glucose
Resting PMN	6900	1250
+ Control-Sepharose	7400	1215
+ C3-Sepharose	32 900	1735

The incubation mixture contained 1×10^7 PMN, 23×10^4 Sepharose beads, 0.2 mg glucose, 0.5 μCi of glucose-1-[^{14}C] or 3.6 μCi of glucose-6-[^{14}C]. The basic medium was balanced Hank's solution. Final volume 2 ml. Temperature 37°C. The center well of the flask contained 0.25 ml of 20% KOH. After 15 min the reaction was stopped by addition of 2 ml of 0.5N H_2SO_4. After additional 30 min the content of the center well was transferred into vials for scintillation counting and counted in a LS-100 Beckman scintillation counter. Bray's solution was used as the scintillation cocktail [23].

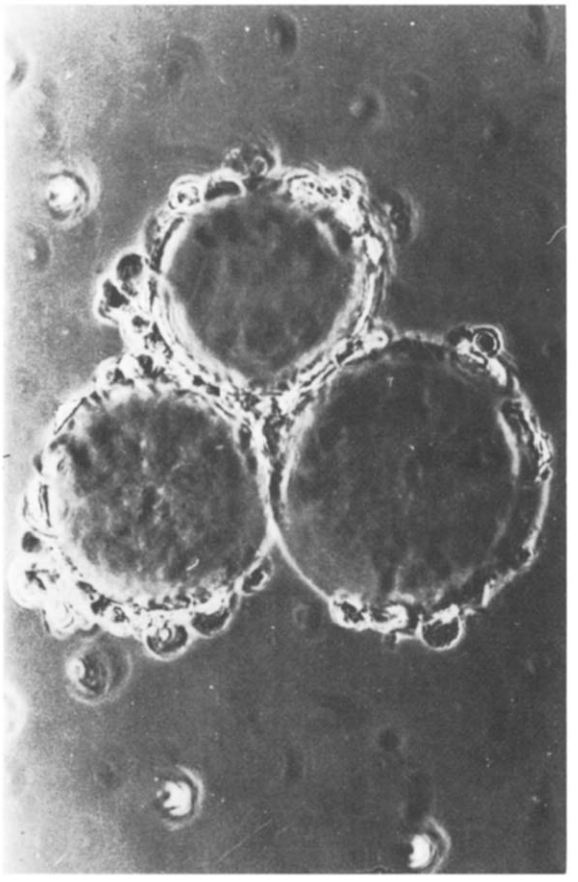

Fig.1. Rosette appearance of a mixture of human polymorphonuclear leucocytes and C3-Sepharose. 1×10^6 PMN were mixed with $40–50 \times 10^3$ beads in 0.2 ml at 37°C for 10–15 min. The suspension was then examined under phase microscope.

Microscopic examination of a mixture of PMN and Sepharose revealed cell adherence to the beads covered with either C3 (fig.1) of Con A (not shown) in a typical rosette appearance. No adherent leucocytes to control Sepharose were detected.

The change in PMN activity induced by the interaction between C3-Sepharose and leucocytes was also studied at different cell/beads ratios. As shown in fig.2 by increasing the amount of beads available to leucocytes a parallel enhancement of $^{14}CO_2$ production from 1-[^{14}C]glucose was detected.

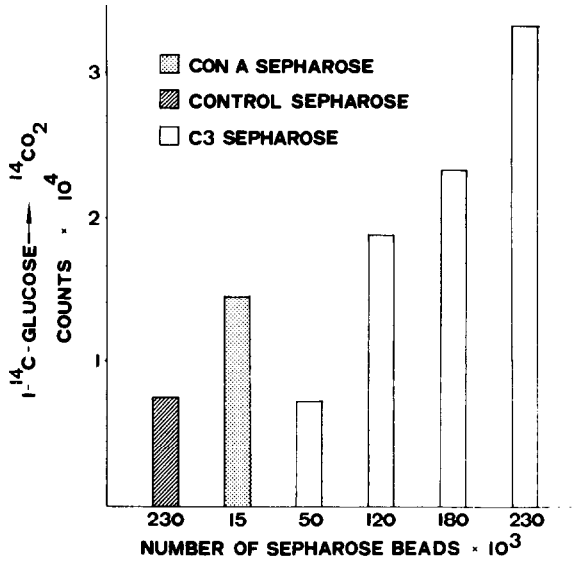

Fig.2. Effect of varying amount of C3-Sepharose on the oxidation of 1-[^{14}C]glucose by human polymorphonuclear leucocytes and a comparison between the effect of C3-Sepharose and that of Con-A-Sepharose on glucose oxidation. For details see the footnote to table 1.

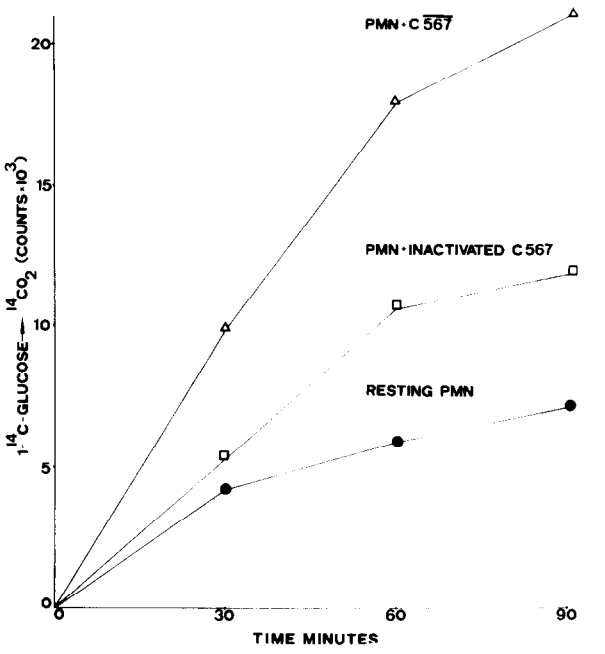

Fig.3. Effect of C567 and inactivated C567 on the oxidation of 1-[^{14}C]glucose by human polymorphonuclear leucocytes. C567 was inactivated by heating at 56°C for 30 min. The amount of C567 of inactivated C567 that was used corresponded to 50 μg protein. Other experimental details as in the footnote to table 1 except that 5 × 10^6 cells were used in each assay.

In order to gain informations about the relationship between the stimulation of HMS activity and the adhesion of leucocytes we compared the metabolic activation of leucocytes challenged with C3-Sepharose or Con A-Sepharose. By using the same amount of beads coated with either C3 or Con A the number of leucocytes adherent to the two types of beads was similar whereas the stimulation induced by Con A-Sepharose was greater than that induced by C3-Sepharose. Accordingly (fig.2), a significant increase of 1-[^{14}C]glucose oxidation could be obtained only by using about 10 × 10^4 C3-Sepharose beads as compared to the small number (15 × 10^3) of Con A-Sepharose beads that gave a two fold increase in glucose oxidation.

Fig.3 shows that C567 markedly stimulated the oxidation of 1-[^{14}C]glucose whereas inactivated C 567 had only a slight effect.

4. Discussion

The plasma membrane of leucocytes plays an important role in regulating the metabolic behaviour and several other functions of these cells such as the recognition of foreign material, the endocytosis and the exocytosis [18]. Among the various agents that are able to interact with the plasma membrane of leucocytes, thus causing metabolic stimulation of the cell and selective release of granule enzymes, the complement is particularly interesting in view of its biological importance. The complement fragment C5a has been shown to be able to stimulate both the activity of the hexose monophosphate shunt [9] and the exocytosis [10]. The latter function of PMN can also be activated by C3a and C567 [19].

This paper demonstrates that the interaction of C3b or C567 with human PMN can also stimulate the metabolic activity of these cells. It is known that hydrogen peroxide (H_2O_2) and superoxide ($O_2^-\cdot$) are produced during stimulation of the aerobic metabolism of leucocytes [3,4,18]. These molecules play a key role in the oxidative damage to the surrounding

tissue and to leucocytes themselves. In addition the metabolic stimulation of leucocytes is usually accompanied by release of granule constituents, that are regarded as the effectors of tissue injury at the site of the inflammatory process [20]. We have recently found that leucocyte granules also contain a factor that activates the complement through the alternate pathway (Tedesco et al., in preparation). Therefore the presence of the activated complement at the inflammatory site may trigger leucocytes to initiate, even in absence of phagocytosable material, a vicious circle that is likely to play a relevant role in propagating the inflammation. The fragment C3b or other biologically active fragments, originating from activation of the complement through the classical pathway or the alternate pathway, would stimulate the oxidative metabolism of PMN and the release of granule constituents, including the complement activating factor, which, in turn, would cause further activation of the complement through the alternate pathway.

The use of immobilized C3b on the surface of non phagocytosable Sepharose beads to stimulate leucocytes is also interesting from a different point of view. It is maintained that the respiratory stimulation of phagocytes challenged with phagocytosable particles is independent of ingestion per se, but is rather related to a perturbation of the plasma membrane of phagocytes following contact with the particles [21,22]. This concept has been emphasized by the results obtained in this and in other laboratories using artificial models that made it possible to stimulate leucocytes with agents that only interact with the constituents of the plasma membrane [5,8,24,25]. The activation of glucose oxidation in leucocytes that adhere to C3-Sepharose by means of specific receptor sites for C3b is a further demonstration that the signal for the metabolic stimulation of PMN is generated by the adherence of these cells to the substrate regardless whether this is phagocytosable or not.

The mechanism whereby this regulatory function is exerted is unknown. The finding that the same number of Sepharose beads coated with either Con A or C3b elicited a quantitatively different response, despite an equal number of PMN adhered to the beads, indicates that different receptor sites on the leucocyte surface are not equivalent as 'triggers' of the metabolic stimulation. A different threshold of perturbability of the various molecules of the cell membrane would enable the cell to modulate its response according to the quality of the environmental stimuli.

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