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De Novo Synthesis of Diacylglycerol from Glucose

A NEW PATHWAY OF SIGNAL TRANSDUCTION IN HUMAN NEUTROPHILS STIMULATED DURING PHAGOCYTOSIS OF β-GLUCAN PARTICLES*

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The phagocytosis of β-glucan particles by human neutrophils and the associated activation of NADPH O_2^- forming oxidase were accompanied by an increased hydrolysis of phosphoinositides by phospholipase C, hydrolysis of phosphatidylcholine by phospholipase D, accumulation of diglyceride (DG) mass, and $[Ca^{2+}]_i$ rise. The reaction of phospholipid hydrolysis played a minor role in the formation of DG, which was mainly formed by *de novo* synthesis from glucose. The activation of this pathway was shown by the stimulation of the incorporation of [U-^{14}C]glucose into DG, which occurred very rapidly after the challenge of neutrophils with β-glucan particles. This DG derived from glucose was found almost completely as 1-acyl-2-acyl-glycerol (DAG). On the basis of the finding that phosphatidic acid was the precursor of DAG, an increase in the incorporation of [U-^{14}C]acetate into DAG did not occur, and the [^{14}C]radioactivity was in the glycerol backbone, the synthesis of DAG from [U-^{14}C]glucose occurred very likely via dihydroxyacetone phosphate and glycerol 3-phosphate, stepwise acylation to phosphatidic acid, and dephosphorylation by phosphatidate phosphatase.

β-Glucan particles are ingested by phagocytes and stimulate the respiratory burst (1, 2). The recognition sites of β-glucan, a β-linked branched chain polysaccharide of glucose subunits, are lectins of the phagocyte surface (3). Among these lectins it has been proposed, on the basis of studies using monoclonal antibody to CD11/18 complex, that α-chain of complement receptor CR3 is able to bind β-glucan and activate signals for ingestion and the respiratory burst (2, 4). Here, we addressed the problem of the transmembrane signaling triggered by β-glucan particles. Our results provide evidence that, besides an increase in phosphoinositide turnover, $[Ca^{2+}]_i$ and hydrolysis of phosphatidylcholine by phospholipase D, β-glucan induced a *de novo* synthesis of DAG[1] from glucose. This synthesis occurred via dihydroxyacetone phosphate and glycerol 3-phosphate, stepwise acylation to phosphatidic acid, and dephosphorylation by phosphatidate phosphohydrolase. To our knowledge this is the first demonstration that a recognition system of cell surface triggers a rapid stimulation of *de novo* synthesis of the lipidic second messenger DAG from glucose in phagocytes.

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[1] The abbreviations used are: DAG, 1-acyl-2-acyl-glycerol; EAG, 1-*O*-alkyl-2-acyl-glycerol; DG, diglyceride; alkyl-PEt, 1-*O*-alkyl-phosphatidylethanol; PA, phosphatidic acid; alkyl-PA, 1-*O*-alkyl-phosphatidic acid; alkyl-DG, 1-*O*-alkyl-diglyceride; Hepes, 4(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

MATERIALS AND METHODS

Reagents—Bovine cardiolipin, octyl-β-glucoside, 1,2-dioleoyl-glycerol, propranolol, and Baker's yeast-derived glucan were purchased from Sigma. Quin 2/AM were from Calbiochem (La Jolla, CA). AG-1-X8 resin was from Bio-Rad. *myo*-[2-^{3}H]Inositol (10–20 Ci/mmol), [γ-^{32}P]ATP (specific activity 3,000 Ci/mmol), 1-*O*-[^{3}H] octadecyl-*sn*-glycero-3-phosphocholine (110 Ci/mmol), [U-^{14}C]glucose (256 mCi/mmol), [U-^{14}C]acetic acid, sodium salt (55 mCi/mmol) were purchased from Amersham Corp. *sn*-1,2-Diacylglycerol kinase was purchased from Lipidex, Inc. (Wesfield, NJ), and Rhizopus lipase was from Boehringer Mannheim.

Neutrophil Preparations—Human neutrophils were prepared from venous blood of healthy donors as in Ref. 5 and suspended in Hank's balanced salt solution containing 0.5 mM $CaCl_2$, 20 mM Hepes, and 5.6 mM glucose (pH 7.4).

Preparation of β-Glucan Particles—Baker's yeast-derived glucan particles were prepared as in Ref. 2, suspended in glucose-free Hank's buffer and sonicated (three or four bursts of 100 watts).

Respiratory Burst Assay—The respiratory burst was monitored as O_2 consumption at 37 °C with a Clark oxygen electrode, fitted in a closed plastic chamber using 2×10^7/ml neutrophils as previously described (5).

Phagocytosis Assay—The conditions of incubation in the phagocytic assay were the same used for O_2 consumption. After addition of β-glucan particles (1 mg) to 1 ml of cell suspensions (2×10^7), samples were withdrawn at different times and fixed with 2% glutaraldehyde in Hank's buffer for electron microscopic examination, or 10-fold diluted in cold Hank's buffer containing 2 mM *N*-ethylmaleimide for light microscopic examination as in Refs. 5 and 6.

Changes in Cytosolic Ca^{2+} Concentration—$[Ca^{2+}]_i$ was monitored in neutrophils loaded with 10 μM Quin 2/AM as described in Ref. 5.

Phospholipids Metabolism—The study of the activation of phospholipid metabolism by β-glucan particles was carried out in the same experimental conditions as described above. The activation of phosphoinositide turnover was investigated by measuring the formation of [^{3}H]inositol phosphates in neutrophils prelabeled with *myo*-[^{3}H] inositol as previously reported (5). The activation of phospholipase D was performed in neutrophils prelabeled with [^{3}H]alkyl-lyso-phosphatidylcholine by measuring the formation of [^{3}H]alkyl-PA, [^{3}H] alkyl-DG, and [^{3}H]alkyl-PEt in the absence and presence of 0.5% ethanol as described in Refs. 7 and 8. DG mass was determined enzymatically transforming diglyceride to [^{32}P]phosphatidic acid using [γ-^{32}P]ATP and diglyceride kinase according to Ref. 9. In order to distinguish 1-*O*-alkyl *versus* 1-acyl diglycerides, we have used the procedure described in Ref. 10. Total DG were converted to [^{32}P] phosphatidic acid according to Preiss *et al.* (9). *Rhizopus arrhizus* lipase was then added to decompose selectively 1-acyl-containing species leaving 1-alkyl-2-acyl species unaffected. The resulting [^{32}P] phosphatidic acid and [^{32}P]lyso-phosphatidic acid were separated on thin layer chromatography plates as described in Ref. 5.

The radioactivity remaining in [^{32}P]phosphatidic acid region corresponded to alkyl-acyl-species while that migrating in [^{32}P]lyso-phosphatidic acid region corresponded to diacyl species.

Formation of [^{14}C]DG from Glucose—The incorporation of [U-^{14}C] glucose into DG was investigated by adding 2 μmol of [U-^{14}C]glucose (6 μCi) to 2 × 10^7 neutrophils suspended in 1 ml of glucose-free Hank's buffer, 1 min before the challenge with β-glucan particles. At indicated times, samples were withdrawn, and lipids were extracted (11). [^{14}C]DG and [^{14}C]PA were separated on Silica Gel G plates as reported in Ref. 7 and quantified by liquid scintillation spectrometry. The average specific activity of [^{14}C]glucose (dpm/nmol) was 7,496 ± 781 in 10 different experiments.

In order to distinguish the ^{14}C radioactivity in glycerol backbone and in fatty acids the spots of [^{14}C]DG were scraped off and digested at 65 °C for 2 h in closed tubes containing 5% KOH MeOH as in Ref. 12. After the mixtures were neutralized with HCl, lipids were extracted and two phases separated. The upper aqueous phase and lower $CHCl_3$ phase were assayed for radioactivity by liquid scintillation spectrometry.

RESULTS AND DISCUSSION

Fig. 1 reports the time course of phagocytosis by human neutrophils of β-glucan particles and associated activation of O_2 consumption. In our conditions the phagocytic process started very early, reached the maximum value within 2–3 min, and then ceased. The respiratory burst paralleled the ingestion.

The challenge of neutrophils with β-glucan particles is followed by a rapid stimulation of phosphoinositide hydrolysis, measured as [^{3}H]inositol phosphates formation in cells labeled with *myo*-[^{3}H]inositol and by an increase in $[Ca^{2+}]_i$ (Fig. 2). It has been recently demonstrated that the activation of neutrophils with formylmethionylleucylphenylalanine, phorbol esters, or Ca^{2+} ionophores is accompanied by a stimulation of hydrolysis of phosphatidylcholine by phospholipase D, with formation of PA, DAG, and EAG (7, 8, 13–20). The data reported in Fig. 3*A* show that the phospholipase D activity was stimulated also with β-glucan particles. In fact, the challenge with these particles of neutrophils labeled with [^{3}H]alkyl-lyso-phosphatidylcholine caused a very rapid increase in the formation of [^{3}H]alkyl-PA and [^{3}H]alkyl-DG. Moreover, the former is produced more rapidly than the second as expected if [^{3}H]alkyl-DG was derived from dephosphorylation of [^{3}H]alkyl-PA. In the presence of 0.5% ethanol, the stimulation of [^{3}H]alkyl-PA and [^{3}H]alkyl-DG formation was greatly inhibited (Fig. 3*B*) because phospholipase D catalyzed the transphosphatidylation reaction with formation of [^{3}H]alkyl-PEt (16, 21).

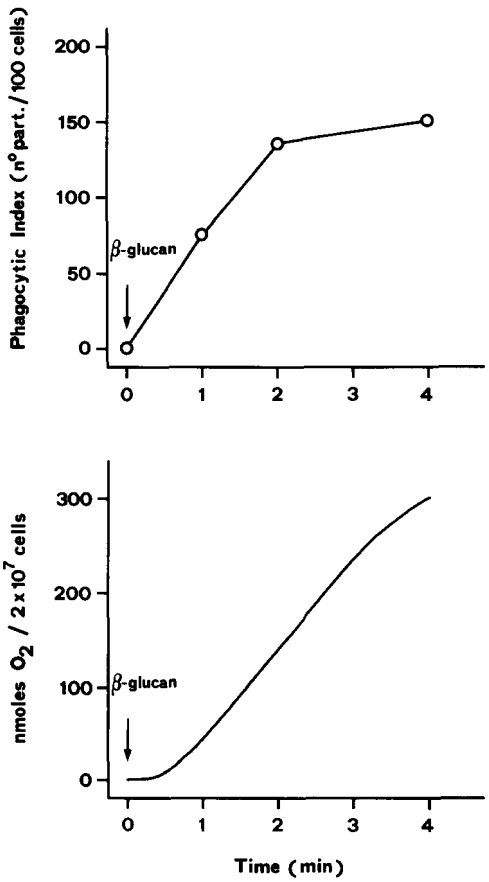

FIG. 1. **Time course of phagocytosis of β-glucan particles in human neutrophils and associated respiratory burst.** Neutrophils (2 × 10^7/ml) were stimulated with 1 mg/ml of β-glucan particles. Results of one representative of five independent experiments are reported.

Fig. 4 shows that the activation of neutrophils by β-glucan particles was also associated with a marked formation of DG mass. This lipid can be derived either directly from hydrolysis of phosphoinositides or other phospholipids by phospholipase C or from dephosphorylation of PA produced by phospholipase D. In order to characterize the source of DG we have used propranolol, a drug that inhibits the phosphatidate phosphohydrolase (22). In neutrophils treated with this drug, the formation of DG mass by β-glucan particles was markedly inhibited (Fig. 4) indicating that the main source of DG was PA.

It has been already reported that in neutrophils stimulated by fMLP, the increased formation of PA is mainly due to the hydrolysis of phosphatidylcholine by phospholipase D (7, 16, 18). In order to establish whether or not the phospholipase D was the main reaction forming PA also in neutrophils stimulated by β-glucan particles, we have investigated the effect of ethanol. The data reported in Fig. 4 show that in the presence of this alcohol the production of DG mass by β-glucan was unchanged, while that of [^{3}H]alkyl-PA and [^{3}H]alkyl-DG was greatly inhibited (Fig. 3*B*). This clearly demonstrates that PA derived from the activity of phospholipase D was not the main source of DG.

This unexpected finding prompted us to identify an alternative pathway responsible for the increase in DG formation compatible with the finding that the main precursor is PA. It is known that DG can be synthesized from glucose via dihydroxyacetone phosphate, glycerol 3-phosphate, with stepwise acylation to PA followed by dephosphorylation to DG (23–29). This process can be investigated by measuring the incorporation of [U-^{14}C]glucose into DG (24, 26). We have performed these experiments and the results reported in Fig. 5 show that indeed the interaction of neutrophils with β-glucan particles stimulated the incorporation of [U-^{14}C]glucose into DG. This was detectable within 30 s and progressively increased throughout the period of observation. On the basis of the specific activity of [U-^{14}C]glucose, it can be calculated (Table I) that 60% of the DG was derived from exogenous glucose. This figure may have been underestimated due to the dilution of [U-^{14}C]glucose by endogenous glucose 6-phosphate formed by glycogenolysis. It is known that the activation of leukocytes during phagocytosis is accompanied by a stimulation of glycogenolysis (30, 31), but we have not quantified this process in the present work. On the basis of the data reported by others (30), in human neutrophils glycogenolysis increased during phagocytosis by about 60%, and this value could account for the dilution of [U-^{14}C]glucose in the experiments reported in Table I. Whichever is the precise figure, it can be concluded that the *de novo* synthesis from glucose represents the main source of DG in neutrophils activated by β-glucan

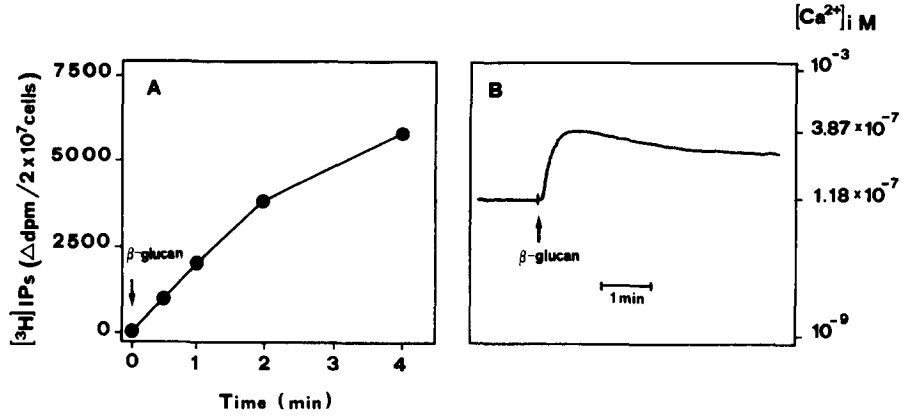

FIG. 2. **Formation of [^{3}H]inositol phosphates (*[^{3}H]IPs*) (*A*) and increase in $[Ca^{2+}]_i$ (*B*) in neutrophils challenged with β-glucan particles (1 mg/ml).** Basal level of [^{3}H]inositol phosphates was 4,235 dpm/2 × 10^7 cells. The results of *A* and *B* are of one experiment representative of four.

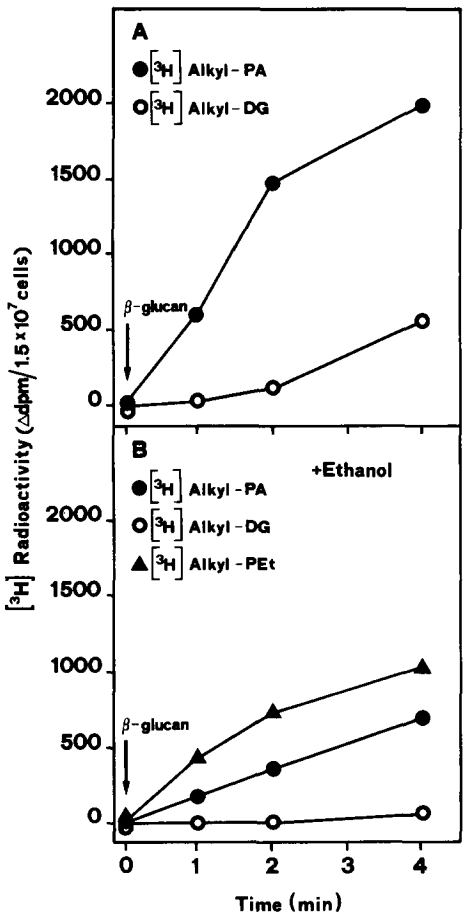

FIG. 3. **Kinetics of [^{3}H]alkyl-PA, [^{3}H]alkyl-DG, [^{3}H]alkyl-PEt formation by β-glucan particles (1 mg/ml) in the absence (*A*) and presence (*B*) of 0.5% ethanol.** Data are of one experiment representative of five. Basal values of [^{3}H]alkyl-PA, [^{3}H]alkyl-DG, and [^{3}H]alkyl-PEt were 925, 1,070, and 312 dpm in the presence and 880 and 932 dpm in the absence of ethanol, respectively.

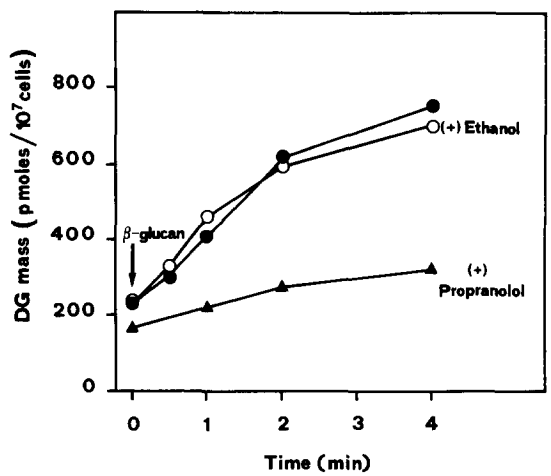

FIG. 4. **Production of DG mass in human neutrophils challenged with β-glucan particles (1 mg/ml).** The mass of [^{32}P]PA was determined from the known specific activity of [γ-^{32}P]ATP. Where indicated 0.5% ethanol or 250 μM propranolol were added 5 min before the stimulant. The results are of one experiment representative of six.

particles. When the experiments were performed in the presence of propranolol, the incorporation of [U-^{14}C]glucose into DG was completely suppressed, while concomitantly [^{14}C]PA accumulated, confirming that most of the [^{14}C]DG was derived from [^{14}C]PA (Fig. 5). Furthermore, the finding that in the absence of propranolol the formation of [^{14}C]PA was much smaller than that of [^{14}C]DG indicated that the rate of PA production from glucose was similar or only slightly higher than that of its dephosphorylation by PA phosphohydrolase.

It is known that the stimulation of neutrophils with different agonists causes the generation of DAG and EAG. Using the procedure described by Tyagi *et al.* (10), we have investigated if the [^{14}C]DG, identified on the basis of the migration on thin layer chromatography with the standard of 1,2-oleoylglycerol, also contained EAG that comigrates with DAG. The lipid extract from neutrophils after phosphorylation of DG to the corresponding [^{32}P]PA according to Preiss *et al.* (9) was subjected to hydrolysis by lipase from *Rhizopus arrhizus* that specifically degrades the 1-acyl-containing species (DAG) but not the 1-alkyl containing species (EAG). The results of two different experiments reported in Table II indicate that DG found after stimulation with β-glucan particles was DAG. Control experiments performed with the same cell populations have shown that DG produced 4 min after stimulation with 100 nM fMLP was about 60% DAG and 40% EAG. The very small amount of EAG, which derived from phospholipase D, confirmed the negligible role of this enzyme in the formation of DAG in neutrophils stimulated by β-glucan particles.

The increased synthesis of [^{14}C]DG from [^{14}C]glucose in neutrophils challenged with β-glucan particles could be due to acylation of [^{14}C]dihydroxyacetone phosphate or [^{14}C]glycerol 3-phosphate by endogenous unlabeled fatty acids or by ^{14}C-fatty acids formed from [^{14}C]glucose through [^{14}C]acetyl-CoA. In order to answer this question we have subjected the [^{14}C]DAG spot separated by thin layer chromatography to

alkaline methanolysis (see "Materials and Methods"). After extraction of the product of hydrolysis, the ^{14}C radioactivity was recovered in the aqueous phase and not in $CHCl_3$ phase. This demonstrated that the ^{14}C radioactivity was in the glycerol backbone of DAG and not in the fatty acids, thus excluding the possibility that the *de novo* synthesis of [^{14}C]DG was due to increased acylation by fatty acids derived from [^{14}C]glucose. This was confirmed also by the finding that the challenge of neutrophils with β-glucan particles was not followed by an increase in the incorporation of [^{14}C]acetate into DAG (data not shown).

The activation of *de novo* synthesis of diacylglycerol by β-glucan particles could be due to an increased flux of glucose through glycolysis as occurred in myocytes treated with insulin (28), and in pancreatic islets (24, 26), endothelial, and smooth muscle cells with high glucose concentration (27). Comparing the effect of propranolol, the inhibitor of phosphatidate phosphohydrolase, on the formation of [^{14}C]PA from [^{14}C]glucose in resting and β-glucan-activated neutrophils, we have found (Fig. 5) that the accumulation of [^{14}C]PA was enormously higher in activated than in resting cells. This result demonstrated that β-glucan particles triggered an increase in the formation of [^{14}C]PA from glucose followed by continuous dephosphorylation to [^{14}C]DAG, but did not clarify if this increase was due to a stimulation of glucose flux through glycolysis or of the rate of the reactions forming PA from dihydroxyacetone phosphate (25). This problem remains to be investigated further. It has been previously shown in our (32) and other laboratories (30, 33) that in neutrophils the phagocytosis of particles different from β-glucan is associated with a slight increase of glucose consumption and lactate formation (plus 10–20%).

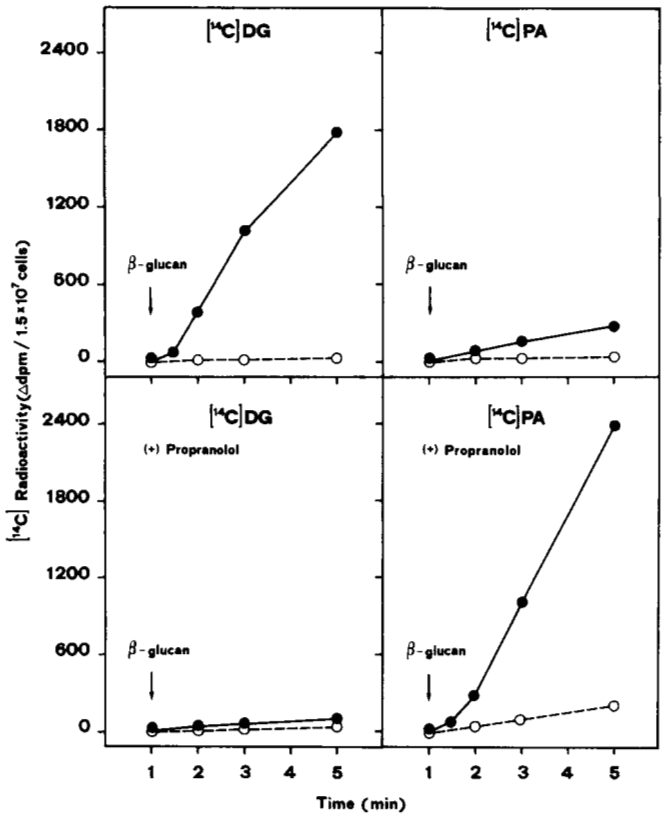

FIG. 5. **Time course of [U-^{14}C]glucose incorporation into DG and PA in neutrophils challenged with β-glucan particles (1 mg/ml).** The results are of one experiment representative of 10 in the absence and six in the presence of 250 μM propranolol. ●—●, in the presence of β-glucan particles. O– – –O, control. In six experiments the formation of DG mass was also measured (see Fig. 4). For details and specific activity of [U-^{14}C]-glucose see "Materials and Methods."

The stimulated incorporation of [U-^{14}C]glucose into DAG, the ingestion of β-glucan particles, and the associated respiratory burst were insensitive to 100 nM staurosporine, a dose that in our experimental conditions markedly depressed the respiratory burst by 30 nM phorbol 12-myristate 13-acetate, and to pretreatment with pertussis toxin, which suppressed completely the respiratory burst by 100 nM fMLP (data not shown). These findings indicate that protein kinase C and the pertussis toxin-sensitive G-protein are not involved in the responses to β-glucan.

Taken together the data presented in this work lead to the conclusion that the *de novo* synthesis from glucose represents the main source of the DAG in neutrophils activated by β-glucan particles. This finding raises a series of problems concerning the receptor involved, the intracellular site of DAG synthesis, the fatty acid composition of this DAG, the functional relations with the cell responses triggered by β-glucan particles, and the effect of the activation of other neutrophil receptors. Regarding the last point, our preliminary experiments have shown that the *de novo* synthesis of [^{14}C]DAG from [U-^{14}C]glucose was not stimulated by fMLP and during phagocytosis of yeast opsonized with C3b/bi. Considering the proposed similarity of the receptor for β-glucan and C3b/bi, this last result deserves to be investigated further.

A last and more general problem raised by the results presented here concerns the role of the rapid activation of DAG synthesis from glucose shown in this paper. Owing to the function of DAG as second messenger, this signaling pathway could be a mechanism by which recognition systems

TABLE I

Formation of DG mass and incorporation of [U-^{14}C]glucose into DG in neutrophils activated during phagocytosis of β-glucan particles

The experimental conditions were those described in Fig. 5. Specific activity of [U-^{14}C]glucose was 7,131 ± 746 dpm/nmol. Values are for 1.5 × 10^7 cells and are expressed as differences between stimulated with β-glucan particles and control neutrophils. The basal values of DG mass and [^{14}C]DG in control neutrophils were 343 ± 68 pmol and 26 ± 10 dpm in the absence and 245 ± 31 pmol and 38 ± 15 dpm in the presence of propranolol, respectively. The diacylglycerol mass and [^{14}C]DG were measured on the same batches of neutrophils. Values are means ± S.D. of the experiments in brackets.

Additions	Time	DG mass	[^{14}C]DG	[U-^{14}C]glucose equivalents	[^{14}C]DG	% DG from [^{14}C]glucose
	min	*pmol*	*dpm*	*pmol*	*pmol*	
β-Glucan particles (1 mg/ml)	2	491 ± 81	835 ± 123	118 ± 14	236 ± 28	48 (6)
	4	714 ± 128	1,483 ± 385	210 ± 42	420 ± 84	59 (6)
β-Glucan particles + propranolol	2	131 ± 42	<70	<10		(4)
	4	205 ± 58	<70	<10		(4)

TABLE II

Quantitation of EAG and DAG in DG produced in neutrophils activated during phagocytosis by β-glucan particles and fMLP

Human neutrophils (2×10^7/ml) were stimulated for 4 min with β-glucan particles or fMLP. Total DG produced by neutrophils were processed and EAG species and DAG species determined according to Ref. 10 (see "Materials and Methods"). The stimulation with fMLP was carried out in neutrophils pretreated (5 min) with cytochalasin B (5 μg/ml).

Addition	Experiment no.	pmol/10^7 cells	
		DAG	EAG
None	1	176	45
	2	162	53
β-Glucan particles (1 mg/ml)	1	606	59
	2	699	66
fMLP (100 nM)	1	635	372
	2	709	414

of cell surface transduce external signals into cellular functions.

REFERENCES

1. Williams, J. D., Topley, N., Alobaidi, H. M., and Harber, M. J. (1986) *Immunology* **58,** 117–124
2. Ross, G. D., Cain, J. A., Myones, B. L., Newman, S. L., and Lachmann, P. J. (1987) *Complement* **4,** 61–74
3. Sharon, N., and Lis, H. (1989) *Science* **246,** 227–246
4. Cain, J. A., Newman, S. L., and Ross, G. D. (1987) *Complement* **4,** 75–86
5. Rossi, F., Della Bianca, V., Grzeskowiak, M., and Bazzoni, F. (1989) *J. Immunol.* **142,** 1652–1660
6. Sbarbati, A., Zancanaro, C., Franceschini, F., Balercia, G., Morroni, M., and Osculati, F. (1990) *Am. J. Anat.* **188,** 199–211
7. Billah, M. M., Eckel, S., Mullmann, T. J., Egan, R. W., and Siegal, M. I. (1989) *J. Biol. Chem.* **264,** 17069–17077
8. Rossi, F., Grzeskowiak, M., Della Bianca, V., Calzetti, F., and Gandini, G. (1990) *Biochem. Biophys. Res. Commun.* **168,** 320–327
9. Preiss, J., Loomis, C. R., Bishop, W. R., Stein, R., Niedel, J. E., and Bell, R. M. (1986) *J. Biol. Chem.* **261,** 8597–8600
10. Tyagi, S. R., Burnham, D. N., and Lambeth, J. D. (1989) *J. Biol. Chem.* **264,** 12977–12982
11. Bligh, E. G., and Dyer, W. J. (1957) *Can. J. Biochem. Physiol.* **37,** 911–917
12. Ritthenhouse-Simmons, S. (1979) *J. Clin. Invest.* **63,** 580–587
13. Pai, J-K., Siegel, M. I., Egan, R. W., and Billah, M. M. (1988) *Biochem. Biophys. Res. Commun.* **150,** 355–364
14. Paj, J-K., Siegel, M. I., Egan, R. W., and Billah, M. M. (1988) *J. Biol. Chem.* **263,** 12472–12477
15. Cabot, M. C., Welsh, C. J., Cao, H., and Chabbott, H. (1988) *FEBS Lett.* **233,** 153–157
16. Billah, M. M., Pai, J.-K., Mullmann, T. J., Egan, R. W., and Siegel, M. I. (1989) *J. Biol. Chem.* **264,** 9069–9076
17. Gelas, P., Ribbes, G., Record, M., Terce, F., and Chap, H. (1989) *FEBS Lett.* **251,** 213–218
18. Agwu, D. E., McPhail, L. C., Chabot, M. C., Daniel, L. W., Wykle, R. L., and McCall, C. E. (1989) *J. Biol. Chem.* **264,** 1405–1413
19. Dougherty, R. W., Dubay, G. R., and Niedel, J. E. (1989) *J. Biol. Chem.* **264,** 11263–11269
20. Truett, A. P., Snyderman, R., and Murray, J. J. (1989) *Biochem. J.* 909–913
21. Dawson, R. M. C. (1967) *Biochem. J.* **102,** 205–210
22. Koul, O., and Houser, G. (1987) *Arch. Biochem. Biophys.* **253,** 453–461
23. Berne, C. (1975) *Biochem. J.* **152,** 667–673
24. Dunlop, M. E., and Larkins, R. G. (1985) *Biochem. Biophys. Res. Commun.* **132,** 467–473
25. Bell, R. M., and Coleman, R. A. (1980) *Annu. Rev. Biochem.* **49,** 459–487
26. Peter-Riesch, B., Fathi, M., Schlegel, W., and Wollheim, C. B. (1988) *J. Clin. Invest.* **81,** 1154–1161
27. Lee Tian-Shing, Saltsman, K. A., Ohashi, H., and King, G. L. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 5141–5145
28. Farese, R. V., Konda, T. S., Davis, J. S., Standaert, M. L., Pollet, R. J., and Cooper, D. R. (1987) *Science* **236,** 586–588
29. Chiarugi, V., Bruni, P., Pasquali, F., Magnelli, L., Basi, G., Ruggiero, M., and Farnararo, M. (1989) *Biochem. Biophys. Res. Commun.* **164,** 816–823
30. Klebanoff, S. J., and Clark, R. A. (1978) *The Neutrophil: Function and Clinical Disorders,* North-Holland, New York
31. Stjernholm, R. L., and Manak, R. C. (1970) *Res. J. Reticuloendothel. Soc.* **8,** 550–560
32. Rossi, F., and Zatti, M. (1966) *Biochim. Biophys. Acta* **121,** 110–119
33. Sbarra, A. J., and Karnovsky, M. (1959) *J. Biol. Chem.* **234,** 1355–1362