A Glycan-Phosphatidylinositol–Specific Phospholipase D in Human Serum

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A group of proteins anchored to the cell by phosphatidylinositol (PI) has recently been identified. The significance of this new class of membrane anchor is unknown; one possibility is that it facilitates release of the molecule by phospholipases. In fact, phospholipase C enzymes specific for the complex carboxyl-terminal glycolipids of these proteins have been isolated from African trypanosomes and from hepatocyte plasma membranes. This study reports the discovery of a glycan-PI–specific phospholipase D in human serum that cleaves both the membrane form of the variant surface glycoprotein of African trypanosomes and its glycolipid precursor, but not phosphatidylethanolamine, phosphatidylcholine, or phosphatidylinositol. Decay-accelerating factor, another PI-anchored molecule, is also cleaved by the enzyme and converted from a hydrophobic to a soluble protein. The enzyme is Ca^{2+}-dependent, heat labile, and not affected by the inhibitor of serine proteases, phenylmethylsulfonylfluoride. Its function is not known, but the present findings indicate that it participates in the metabolism of glycolipid-anchored membrane proteins.

RECENT STUDIES HAVE REVEALED that a growing number of eukaryotic proteins are anchored in the cell membrane by phosphatidylinositol (PI) (*1*). The glycolipid anchor whose structure is best known is that of the variant surface glycoprotein (VSG) of the African trypanosome. The COOH-terminal amino acid of VSG is covalently bound to ethanolamine, which in turn is coupled to an oligosaccharide that contains galactose, mannose, and a single glucosamine residue; the entire complex is *O*-glycosidically linked to dimyristyl-PI (*2*). In mammalian cells, the Thy-1 antigen (*3*), acetylcholinesterase (*4*), and decay-accelerating factor (DAF) (*5, 6*) appear to be linked to the cell membrane by similar glycolipids.

In addition to facilitating lateral movement of the protein in the lipid bilayer (*7*), the anchor may provide a recognition signal for the specific release of the protein from the membrane (*1*). In support of the latter hypothesis is the finding that VSG lipase, a phospholipase C specific for the glycan-PI, is present in the African trypanosome (*8, 9*). VSG lipase cleaves the membrane form of VSG (mfVSG), removing the dimyristylglycerol anchor, and converting mfVSG to its soluble form, sVSG. A similar glycan-PI–specific phospholipase C has been found in mammalian cells (*10*), but whether this enzyme releases membrane-associated PI-anchored proteins is unknown.

Decay-accelerating factor is a membrane protein that binds to complement fragments C3b or C4b (*11*), inhibiting amplification of the complement cascade on cell surfaces (*12, 13*). Two forms of DAF have been described: a hydrophobic form associated with the membrane of all blood elements, endothelial cells, and epithelial cells, and a hydrophilic form found in urine, saliva, and tears (*14–18*). While trying to determine whether a surface-associated membrane phospholipase was responsible for releasing DAF from the cell, we discovered serendipitously a glycan-PI–specific phospholipase D in human serum. In our initial studies we used HeLa cells as a possible source of the putative enzyme, since they secrete large amounts of DAF into the culture medium (*16*). As a substrate, we used mfVSG labeled to a high specificity activity with [^{3}H]myristic acid (*19*). After incubation with HeLa cells, the culture medium was extracted with *n*-butanol to determine whether the ^{3}H-labeled mfVSG had been cleaved and butanol-soluble radioactivity released. In control experiments, ^{3}H-labeled mfVSG was incubated with medium alone. Unexpectedly, the same amount of butanol-extractable radioactivity was released in the presence or absence of HeLa cells. Additional experiments revealed that fetal calf serum—a common constituent under both conditions—as well as human serum and plasma contained the enzymatic activity.

The butanol-soluble product that resulted from treatment with human serum was analyzed by thin-layer chromatography (TLC). All the radioactivity migrated with dimyristyl-phosphatidic acid (DMPA) but not with dimyristyl-glycerol (DMG), which had been

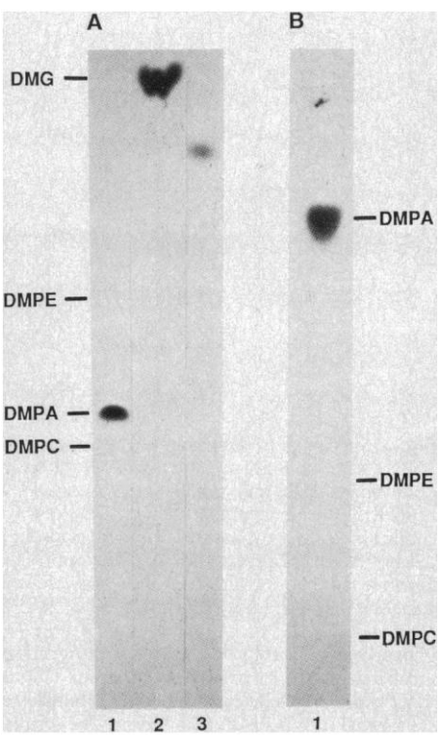

Fig. 1. Identification of cleavage product of the serum enzyme. (**A**) Two micrograms of ^{3}H-labeled mfVSG (4415 dpm/μg, 30 pmol) were treated for 1 hour at 37°C with 1 μl of fresh human serum in 50 m*M* tris-HCl (*p*H 7.4), 10 m*M* NaCl, 2.6 m*M* $CaCl_2$ (buffer 1) or with 0.3 μg of PIPLC in 25 m*M* Hepes-NaOH (*p*H 7.4), containing 0.1% (w/v) sodium deoxycholate. The mixtures contained 0.01% NP-40 and 0.002% SDS introduced with the VSG preparation. The total reaction volume was 100 μl. After incubation, the mixtures were extracted with 1 ml of H_2O-saturated *n*-butanol, the phases separated by centrifugation, and 800 μl of the upper (organic) phase evaporated in Speed-Vac concentrator (*9*). Approximately 60% of the total radioactivity became butanol-soluble after serum treatment. The extracts were resuspended in 40 μl of $CHCl_3$:CH_3OH:H_2O (10:10:3) and spotted on a silica gel, 60 plate (Merck). Adjacent lanes were spotted with 10 μg of lipid standards (Sigma): dimyristyl-phosphatidylcholine (DMPC), dimyristyl-phosphatidylethanolamine (DMPE), dimyristyl-phosphatidic acid (DMPA). The plates were developed with $CHCl_3$:CH_3OH: 0.25% aqueous KCl (55:45:10), air-dried, stained with I_2 to locate lipid standards, sprayed with EnHance (New England Nuclear), and subjected to fluorography. Lane 1, ^{3}H-labeled mfVSG + human serum; lane 2, ^{3}H-labeled mfVSG + PIPLC; lane 3, [9,10-^{3}H]myristic acid (New England Nuclear). The cleavage product chromatographed with DMPA. (**B**) The products of a reaction identical to that in (A), lane 1, were spotted on a silica gel plate and developed with $CHCl_3$:CH_3OH:CH_3COCH_3:CH_3COOH:H_2O (30:10:40:10:5). After migration of the solvent front, the plate was air-dried, sprayed with phosphomolybdic acid stain (Sigma) to reveal the lipid standard, and processed as described for (A). Lane 1, ^{3}H-labeled mfVSG + human serum. The cleavage product chromatographed with DMPA.

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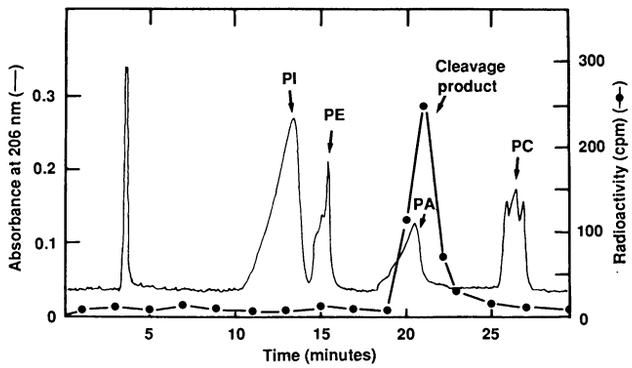

Fig. 2. HPLC analysis of cleavage product. Two micrograms of ^{3}H-labeled mfVSG were treated as previously described with human serum. After incubation and extraction with *n*-butanol, 800 μl of the upper phase was removed and evaporated. The extract was resuspended in an equal volume of hexane:2-propanol:water (6:8:0.75) (solvent A) and subjected to normal-phase HPLC (*37*) on a Microsorb-Silica column (5.0 μm, 4.6 mm by 25 cm; Rainin Instrument Co.) and a Spheri-5 Silica precolumn (2.1 mm by 3 cm; Bronlee). Lipids were eluted with a linear gradient of 100% solvent A at 0 minutes to 100% hexane:2-propanol:water (6:8:1.0) at 15 minutes. HPLC fractions (1.0 ml) were analyzed for radioactivity by liquid scintillation counting after mixing with 0.5 ml of butanol and 10 ml of Hydrofluor. Elution of lipid standards [phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidic acid (PA), and phosphatidylcholine (PC) (Avanti Polar Lipids)] was monitored by ultraviolet absorbance at 206 nm. All radioactivity chromatographed with phosphatidic acid.

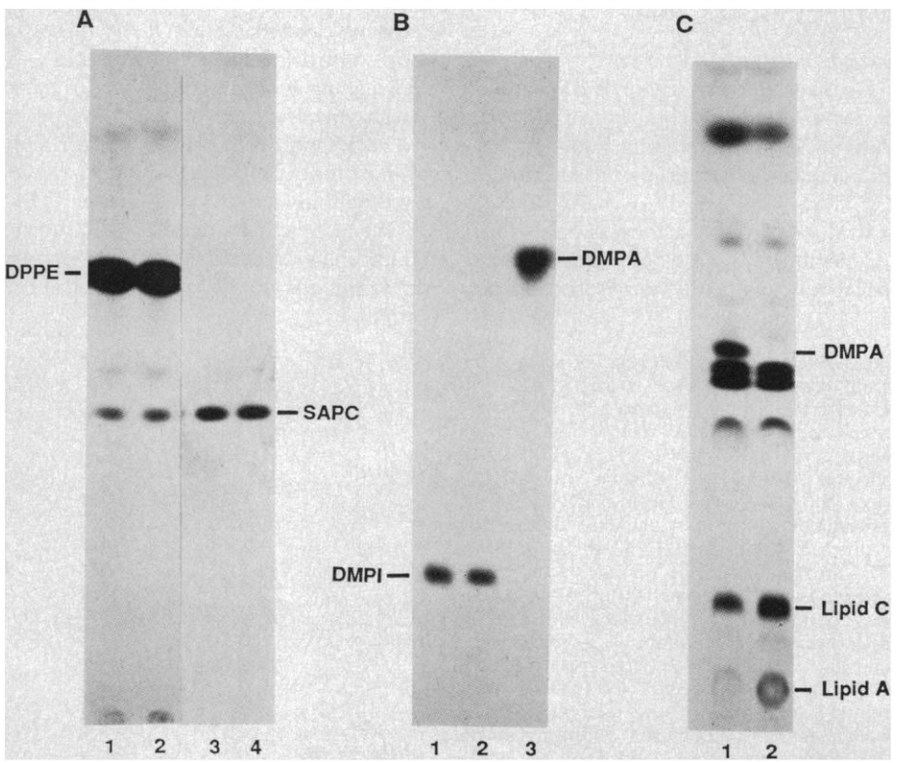

Fig. 3. Specificity of the serum phospholipase D. (**A**) Human serum (1 μl) was incubated for 1 hour at 37°C with L-3-phosphatidylcholine, 1-stearoyl-2-[5,6,8,9,11,12,14,15-^{3}H]arachidonyl (SAPC) (Amersham) (103 mCi/mg, 0.15 pmol) or with L-3-phosphatidylethanolamine, 1,2-di[1-^{14}C]palmitoyl (DPPE) (Amersham) (169 μCi/mg, 130 pmol). Note that DPPE is prepared from L-3-phosphatidylcholine by transphosphatidylation with phospholipase D and contains trace contaminants of phosphatidylcholine and phosphatidic acid. The samples were processed for TLC as described in Fig. 1A. As a negative control, the serum enzyme was inhibited by addition of EGTA (final concentration 25 m*M*). Lane 1, DPPE + human serum; lane 2, DPPE + human serum + EGTA; lane 3, SAPC + human serum; lane 4, SAPC + human serum + EGTA. Neither SAPC nor DPPE was cleaved. (**B**) Dimyristylphosphatidylinositol (DMPI) was prepared by HNO_2 cleavage of ^{3}H-labeled mfVSG (*2*). DMPI was incubated with 1 μl of human serum for 1 hour at 37°C, and the samples were processed for TLC as described in Fig. 1B. Lane 1, DMPI + human serum; lane 2, DMPI + human serum + EGTA; lane 3, ^{3}H-labeled mfVSG + human serum. The serum phospholipase D activity did not cleave DMPI. (**C**) A lipid extract was prepared from [^{3}H]myristate-labeled trypanosomes (*19*), evaporated, and resuspended in 0.1% NP-40 that contained 0.02% SDS. Portions (10 μl) of this preparation were then treated with 1 μl of serum, or with 1 μl of serum + EGTA (final concentration 25 m*M*), for 1 hour at 37°C (total volume 100 μl), and the samples were processed as described in (A). Lane 1, lipid extract + human serum; lane 2, lipid extract + human serum + EGTA. The serum enzyme cleaved lipid A and possibly lipid C, and generated DMPA.

generated by treatment of the substrate with PI-specific phospholipase C (PIPLC) (Fig. 1A). The identity of the product was confirmed by TLC with a different solvent system (Fig. 1B) and by high-performance liquid chromatography (HPLC) (Fig. 2). In both cases, the radioactivity chromatographed with DMPA. These results indicated that the activity present in human serum is a phospholipase D.

The substrate specificity of the serum phospholipase D was examined by incubating it with two known substrates for phospholipase D enzymes (*20*), phosphatidylcholine and phosphatidylethanolamine. Neither phospholipid was cleaved by the serum enzyme (Fig. 3A). Furthermore, the serum enzyme did not act on dimyristyl-phosphatidylinositol (DMPI), a component of the VSG glycolipid (Fig. 3B). A mixture of *Trypanosoma brucei* lipids that had been metabolically labeled with [^{3}H]myristic acid (*21*) was also used as a substrate. Lipid A, the biosynthetic precursor of the VSG glycolipid (*21*), and possibly lipid C, were converted to a product that chromatographed with DMPA (Fig. 3C).

Next, the effects of various reagents on enzymatic activity were examined (Table 1). The phospholipase D activity in human serum is Ca^{2+}-dependent, heat labile, and not inhibited by the serine-esterase inhibitor phenylmethylsulfonylfluoride (PMSF). In addition, the conversion of ^{3}H-labeled mfVSG to DMPA was dependent on both time and serum concentration; after 1 hour of incubation with excess substrate at 37°C under the conditions described in Table 1, 0.1 μl of human serum converted 410 ng of ^{3}H-labeled mfVSG to DMPA.

Because the enzyme is present in human serum, it was important to determine whether a mammalian PI-anchored protein such as DAF is susceptible to cleavage while in its native configuration on the cell membrane. Human lymphocytes, erythrocytes, and HeLa cells were incubated with human serum, and the supernatants were assayed for the presence of DAF by immunoradiometric assay (*15*). DAF was not released from the surface of these cells.

To determine whether the enzyme might cleave isolated membrane DAF, we used a novel assay that discriminated between hydrophobic and hydrophilic forms of DAF. Native purified membrane DAF reincorporates into cell membranes (*13*) and binds tightly to phenyl-Sepharose beads (*6*). Both reincorporation and binding are probably mediated by the diacylglycerol anchor, since DAF-S (DAF treated with PIPLC) does not bind to these beads, nor does it reincorporate into lipid bilayers (*5, 6*). Incubation with human serum resulted in conversion of

approximately 80% of the membrane DAF into a hydrophilic form, that is, a form that did not bind to phenyl-Sepharose (Fig. 4A). Moreover, the enzymatic activity was Ca^{2+}-dependent, not inhibited by PMSF, and heat-sensitive. The polypeptide product of DAF cleavage was further characterized by Western blotting. After treatment with serum, DAF migrated with a slightly smaller M_r (67,000), similar to that of DAF-S (Fig. 4B). The cleavage product was also recognized by antibodies to synthetic peptides representing the NH_2-terminal and the COOH-terminal regions of DAF (Fig. 4B) (*17, 22*).

However, the serum and PIPLC-mediated (DAF-S) cleavage products of DAF were not identical. Only DAF-S was recognized by antibodies to the cross-reacting determinant (CRD), an epitope located within the COOH-terminal carbohydrate structure of many glycolipid-anchored proteins including VSG and DAF (*23, 24*). The CRD epitope is cryptic and revealed only after hydrolysis of the PI anchor with PIPLC or VSG lipase (*9, 10, 23, 24*). The CRD epitope includes the terminal inositol-phosphate, and the presence of phosphate is essential for reactivity with the antibodies (*25*). The lack of reactivity of human serum–treated DAF with antibody to CRD further supports the idea that the enzymatic cleavage generates phosphatidic acid rather than diacylglycerol.

On the basis of these findings, we conclude that human serum contains a glycan-PI–specific phospholipase D, which exhibits similar substrate specificities to both the trypanosomal and mammalian glycan-PI–specific phospholipase C enzymes (*8–10*).

Phospholipase D enzymes have been studied in plants (*20*) as well as in mammalian tissues (*26*), such as rat brain (*27*), heart (*28*), and human eosinophils (*29*). They hydrolyze phospholipids to phosphatidic acid or can catalyze base-exchange of choline or ethanolamine into phospholipids (*26*). For the most part, the phospholipase D activity in plants is Ca^{2+}-dependent (*20*). In mammals, the base-exchange reactions are dependent on Ca^{2+} (*30*), whereas the hydrolytic activity does not require divalent cations (*27, 28*). On the basis of the specificity for the glycolipid anchor and the dependence on Ca^{2+} for activity, the serum enzyme reported here appears to be unrelated to previously known phospholipase D enzymes. Moreover, all of the previously described mammalian phospholipase D enzymes are cell-associated (*27–29*). Low and co-workers have independently found a similar phospholipase D activity in placenta (*31*) and more recently in mammalian plasma (*32*) that converts two other PI-anchored proteins, alkaline phosphatase (*33*) and rat liver 5′-nucleotidase (*34*), from a membrane-bound to a soluble form.

Although the serum enzyme was unable to release DAF from the surface of intact cells, it cleaves detergent-solubilized membrane DAF and generates a hydrophilic form of the protein. Whether the soluble DAF found in HeLa culture supernatants, urine, and plasma (*16*) results from the cleavage of membrane DAF by cell-associated phospholipases is unknown. Nonetheless, it is intriguing that soluble DAF shares properties with serum-cleaved DAF; both have an M_r of 67,000, and both lack the CRD epitope (*24*).

The physiological relevance of the serum phospholipase D remains to be determined, but if the enzyme acts on PI-anchored membrane proteins, it would generate membrane-associated phosphatidic acid. Phosphatidic acid can be produced during receptor-stimulated breakdown of phosphoinositides and has growth factor–like effects; that

Table 1. Effect of various treatments on enzymatic activity. Ten microliters of human serum was incubated under the various conditions in 100 μl of buffer 1 for 1 hour at 37°C. After incubation, the mixtures were extracted with 1 ml of H_2O-saturated *n*-butanol, the phases separated by centrifugation, and 500 μl of the upper (organic) phase analyzed for radioactivity by liquid scintillation counting after mixing with 10 ml of Hydrofluor. Assays were done in duplicate; values represent means. Variation around the mean was always less than 10%. Percentage activity = (cpm after treatment/cpm with no treatment) × 100.

Treatment of serum	Activity (%)
No treatment*	100
EGTA (2.5 m*M*)	0
EGTA (2.5 m*M*) + $CaCl_2$ (1 m*M*)	1.8
EGTA (2.5 m*M*) + $CaCl_2$ (10 m*M*)	170
EGTA (2.5 m*M*) + $MgCl_2$ (1 m*M*)	2.9
EGTA (2.5 m*M*) + $MgCl_2$ (10 m*M*)	27
PMSF (1 m*M*)	80
56°C for 30 minutes	10

*Twenty-seven percent of the total input (2062 cpm or 1.5 μg) was converted to DMPA.

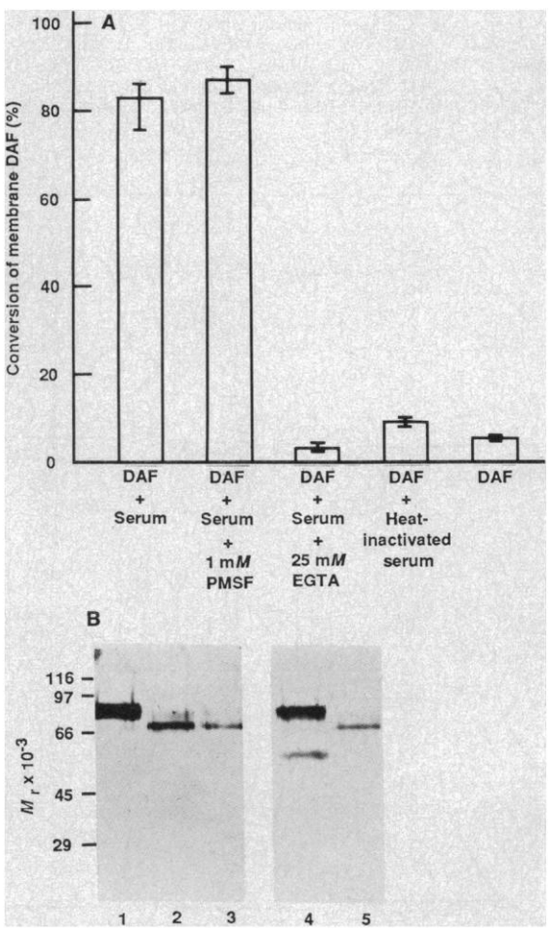

Fig. 4. Cleavage of DAF. (**A**) Purified membrane DAF (0.43 μg) from human erythrocytes (*22*) was incubated for 1 hour at 37°C with 0.1 μl of human serum in buffer 1 (total volume 100 μl) containing 0.01% NP-40. After incubation, 900 μl of phosphate-buffered saline (PBS) was added to the reaction mixture, and then 75 μl of phenyl-Sepharose beads (Pharmacia), which had previously been washed in PBS containing 0.001% NP-40. After incubation for 1 hour at room temperature, the beads were removed by centrifugation, and the supernatants were assayed for DAF by means of a two-site immunoradiometric assay (*15*). The percentage conversion of membrane DAF was calculated as 100× the DAF in the supernatant (in micrograms) divided by 0.43 μg. In control preparations, DAF was incubated with human serum + 25 m*M* EGTA, human serum + 1 m*M* PMSF or heat-inactivated human serum. Results represent the means and range of three experiments. Approximately 80% of the purified membrane DAF was converted to a soluble form by incubation with human serum. Conversion was Ca^{2+}-dependent and not inhibited by addition of PMSF; furthermore, heat treatment of the serum destroyed the enzymatic activity. (**B**) Serum-treated DAF prepared as described in (A) was purified by incubation with CNBr-Sepharose beads (Pharmacia) conjugated with monoclonal antibodies to DAF (*24*). This preparation together with purified intact membrane DAF (*22*), or DAF-S (*5*) were subjected to SDS–polyacrylamide gel electrophoresis under reducing conditions, transferred electrophoretically to nitrocellulose filters, and blotted with antibodies to synthetic peptides representing known sequences of DAF. Antigens: lanes 1 and 4, membrane DAF, lanes 2 and 5, serum-treated DAF; lane 3, DAF-S. Antibodies: lanes 1 to 3, antibodies to synthetic peptide 1 (representing the NH_2-terminal sequence of DAF), lanes 4 and 5, antibodies to synthetic peptide 3 [representing a sequence close to the COOH-terminus of membrane DAF (*17, 22*)]. Serum-treated DAF reacted with both antibodies to peptide 1 and 3. Additionally, serum-treated DAF migrated with DAF-S.

is, it releases Ca^{2+} from intracellular stores, induces expression of c-*fos* and c-*myc* protooncogenes, and stimulates DNA synthesis (*35*). Two PI-anchored molecules, Thy-1 and T cell–activating protein (TAP), are known to be involved in cell activation (*36*). Thus the cleavage of PI-anchored proteins by a serum phospholipase D and its possible effects on membrane signal transduction deserve further investigation.

REFERENCES AND NOTES

1. G. Cross, *Cell* **48**, 179 (1987); M. G. Low, *Biochem. J.* **244**, 1 (1987).
2. M. A. J. Ferguson, M. G. Low, G. Cross, *J. Biol. Chem.* **260**, 14547 (1985).
3. M. G. Low and P. W. Kincade, *Nature (London)* **318**, 62 (1985); A. G. D. Tse, A. N. Barclay, A. Watts, A. F. Williams, *Science* **230**, 1003 (1986).
4. A. H. Futerman *et al.*, *Biochem. J.* **226**, 369 (1985); W. L. Roberts and T. L. Rosenberry, *Biochem. Biophys. Res. Commun.* **133**, 621 (1985); R. Taguchi, K. Suzuki, T. Nakabayashi, I. Ikezawa, *J. Biochem. (Tokyo)* **96**, 437 (1984); M. G. Low and J. B. Finean, *FEBS Lett.* **82**, 143 (1977).
5. M. A. Davitz, M. G. Low, V. Nussenzweig, *J. Exp. Med.* **163**, 1150 (1986).
6. M. E. Medof, E. I. Walter, W. L. Roberts, R. Haas, T. L. Rosenberry, *Biochemistry* **25**, 6740 (1986).
7. J. Thomas *et al.*, *Biophys. J.* **51**, 522a (1987); A. Ishihara, Y. Hou, K. Jacobson, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 1290 (1987).
8. R. Bulow and P. Overath, *J. Biol. Chem.* **261**, 11918 (1986); J. A. Fox, M. Duszenko, M. A. J. Ferguson, M. G. Low, G. A. M. Cross, *ibid.*, p. 15767.
9. D. Hereld, J. L. Krakow, J. D. Bangs, G. W. Hart, P. T. Englund, *ibid.*, p. 13813.
10. J. A. Fox, N. M. Soliz, A. Saltiel, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2663 (1987).
11. T. Kinoshita, M. E. Medof, V. Nussenzweig, *J. Immunol.* **136**, 3390 (1986).
12. E. M. Hoffman, *Immunochemistry* **6**, 391 (1969); A. J. Nicholson-Weller, J. Burge, D. T. Fearon, P. F. Weller, K. F. Austen, *J. Immunol.* **129**, 184 (1982); M. K. Pangburn, R. D. Schreiber, H. J. Müller-Eberhard, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5430 (1983).
13. M. E. Medof, T. Kinoshita, V. Nussenzweig, *J. Exp. Med.* **160**, 1558 (1984).
14. A. S. Asch, T. Kinoshita, E. A. Jaffe, V. Nussenzweig, *ibid.* **163**, 221 (1985); A. Nicholson-Weller, J. P. March, C. E. Rosen, D. B. Spicer, K. F. Austen, *Blood* **65**, 1237 (1985).
15. T. Kinoshita, M. E. Medof, R. Silber, V. Nussenzweig, *J. Exp. Med.* **162**, 75 (1985).
16. M. E. Medof, E. I. Walter, J. L. Rutgers, D. M. Knowles, V. Nussenzweig, *ibid.* **165**, 848 (1987).
17. I. W. Caras *et al.*, *Nature (London)* **325**, 545 (1987).
18. M. E. Medof *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2007 (1987).
19. J. D. Bangs, D. Hereld, J. L. Krakow, G. W. Hart, P. T. Englund, *ibid.* **82**, 3207 (1985).
20. M. Heller, *Adv. Lipid Res.* **16**, 267 (1978).
21. J. L. Krakow, D. Hereld, J. D. Bangs, G. W. Hart, P. T. Englund, *J. Biol. Chem.* **261**, 12147 (1986).
22. M. A. Davitz, D. Schlesinger, V. Nussenzweig, *J. Immunol. Methods* **97**, 71 (1987).
23. C. Bordier, R. J. Etges, J. Ward, M. J. Turner, M. L. Cardoso de Almeida, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5988 (1986); M. L. Cardoso de Almeida and M. J. Turner, *Nature (London)* **302**, 349 (1983); S. C. Steiger, M. L. Cardoso de Almeida, M. C. Blatter, U. Brodbeck, C. Bordier, *FEBS Lett.* **199**, 182 (1986).
24. M. A. Davitz, A. M. Gurnett, M. J. Turner, M. G. Low, V. Nussenzweig, *J. Immunol.* **138**, 520 (1987).
25. S. Shak *et al.*, in preparation.
26. J. N. Kanfer, *Can. J. Biochem.* **58**, 1370 (1980).
27. M. Saito and J. Kanfer, *Arch. Biochem. Biophys.* **169**, 318 (1975).
28. P. C. Schmid, P. V. Reddy, V. Natarajan, H. O. Schmid, *J. Biol. Chem.* **258**, 9302 (1983).
29. L. A. Kater, E. J. Goetzl, K. F. Austen, *J. Clin. Invest.* **57**, 1173 (1976).
30. M. Saito, E. Bourque, J. Kanfer, *Arch. Biochem. Biophys.* **169**, 304 (1975).
31. A. S. Malik and M. G. Low, *Biochem. J.* **240**, 519 (1986); M. G. Low and A. R. S. Prasad, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **46**, 2110 (1987).
32. M. G. Low and A. R. S. Prasad, in preparation.
33. M. G. Low and D. B. Zilversmit, *Biochemistry* **19**, 3913 (1980).
34. M. G. Low and J. B. Finean, *Biochim. Biophys. Acta* **508**, 565 (1978); R. Taguchi and I. Ikezawa, *Arch. Biochem. Biophys.* **186**, 196 (1978).
35. W. H. Moolenaar *et al.*, *Nature (London)* **323**, 171 (1986).
36. H. Reiser *et al.*, *Cell* **47**, 365 (1986); K. C. Gunter *et al.*, *Nature (London)* **326**, 505 (1987).
37. G. W. S. M. Van Kessel *et al.*, *Biochim. Biophys. Acta* **486**, 524 (1977).

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