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# Anti-idiotypic antibody identifies the structural similarity between the phosphatidylcholine-specific monoclonal antibody and phosphatidylcholine-specific lipid transfer protein

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The polyclonal anti-idiotypic antibody (Anti-Id) has been raised against a monoclonal antibody (mAb) that specifically binds to phosphatidylcholine (PC). The anti-Id bound strongly to PC-specific mAbs, but not to the other mAbs that bind to phosphatidylserine, indicating that the anti-Id recognizes the cross-reactive idiotopes expressed on the PC-specific mAbs. The anti-Id also showed an extensive cross-reaction with the PC-specific lipid transfer protein isolated from bovine liver and inhibited the lipid transfer activity of the protein. These results strongly suggest that the anti-Id recognizes a common structure shared between PC-specific mAbs and the PC-specific lipid transfer protein.

Lipid-protein interaction; Monoclonal antibody; Phosphatidylcholine; Anti-idiotypic antibody; Lipid transfer protein

## 1. INTRODUCTION

Many proteins have been shown to interact specifically with choline-containing glycerophospholipids. The typical examples are the receptor for platelet activating factor [1], PC-specific transfer protein [2], PC-specific lipid translocater protein that is responsible for the transmembrane movement of the lipid [3], 1-alkyl-2-lyso-sn-glycero-3-phosphocholine:acetyltransferase [4], and phospholipase C specific for PC [5]. Although much effort has been focused on understanding the nature of specific lipid-protein interactions, the difficulties of handling these proteins because of their limited aqueous solubility and scarcity in the biological membranes have hampered the progress in this area.

The monoclonal antibody that specifically recognizes choline-containing glycerophospholipids may not only provide valuable information about the lipid-protein interactions, but may also represent a structural temperate for the production of anti-Id that could recognize the cross-reactive structures shared between the antibody and the actual receptor molecules [6]. In the previous study we established a series of mAbs that specifically recognizes PC [7]. One of the mAbs, designated JE-1, showed a reactivity profile similar, in regard to their head group and acyl chain specificity, and effect on the enzyme activities of phospholipases, to that of the PC-specific lipid transfer protein [8]. Though the primary structure of the PC-specific lipid transfer protein has been elucidated [9], the sequence or the structure which is responsible for the interaction with the substrate has not yet been identified. In the present study we produced a cross-reactive anti-Id to a PC-specific mAb and investigated the structural similarity between the antibody molecule and the PC-specific lipid transfer protein.

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*Abbreviations:* mAb, monoclonal antibody; PC, phosphatidylcholine; PS, phosphatidylserine; anti-Id, anti-idiotypic antibody; ELISA, enzyme-linked immunosorbent assay

## 2. MATERIALS AND METHODS

### 2.1. *Chemicals*

Egg yolk PC was prepared by chromatography on Aluminum Oxide Neutral and Iatrobeads. 1-acyl-2-[$^{14}$C]linoleoyl-glycerophosphocholine and glycerol-tri[9,10($n$)-$^{3}$H]oleate was purchased from Radiochemical Center, Amersham, UK.

### 2.2. *Production of anti-Id*

Polyclonal anti-Id to the mAb (JE-1) that specifically binds to PC was generated in New Zealand White female rabbits. 3 rabbits were immunized with 0.5 mg of JE-1 in complete Freund's adjuvant subcutaneously, followed by an injection of the antigen in incomplete Freund's adjuvant 4 weeks later. After the boost, the rabbits were bled every week. Immunoglobulin G (IgG) was isolated from the preimmune and immune sera of the rabbits by ammonium sulfate precipitation and affinity chromatography on a protein A-Sepharose column. The resulting IgG was rendered idiotype specific by two adsorptions over Sepharose 4B coupled to the isotype and allotype matched unrelated mouse monoclonal IgM antibody (kindly provided by A. Suzuki, Tokyo Metropolitan Institute of Medical Science).

### 2.3. *Binding of anti-Id to anti-phospholipid mAbs*

The binding of the anti-Id to anti-phospholipid mAbs was



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measured by enzyme-linked immunosorbent assay (ELISA) as described previously [10]. Anti-Id bound was detected by biotinylated anti-rabbit IgG (ZYMED Laboratories, San Francisco, CA) followed by incubation with peroxidase-conjugated streptavidin (ZYMED Laboratories).

2.4. *Binding of anti-Id to PC-specific lipid transfer protein*

The PC-specific lipid transfer protein was isolated from bovine liver according to the method of Kamp et al. [11] and was purified to homogeneity by gel-filtration column chromatography on TSK G2000SWXL (Tosoh corp. Tokyo, Japan). The purified protein showed a single band with an apparent relative molecular mass of 25 000 on sodium dodecyl sulfate polyacrylamide gel electrophoresis. The binding of the anti-Id to the PC-specific lipid transfer protein was examined by ELISA. Briefly, the plates were coated with 14 ng of the purified PC-specific lipid transfer protein in 50 μl of 0.1 M sodium carbonate buffer, pH 9.6, overnight at 4°C. After the wells were blocked with PBS containing 30 mg/ml of BSA, a serial dilution of anti-Id was added to the well and the mixture was incubated for 2 h at room temperature. For the control, IgG purified from the pre-immune serum was used instead of anti-Id. The anti-Id bound was determined as described in 2.3.

2.5. *Inhibition of the binding of anti-phospholipid mAbs by anti-Id*

The binding of the anti-phospholipid mAbs to the corresponding phospholipid antigens was determined by ELISA as described previously [12]. The inhibition of binding of the anti-phospholipid mAbs by anti-Id was examined as follows. Anti-phospholipid mAbs were preincubated with serial dilutions of anti-Id for 2 h at room temperature. The mixtures were transferred to microtiter wells coated with phospholipid antigens and were incubated for 2 h at room temperature. The anti-phospholipid mAbs bound to the corresponding phospholipid antigens were determined by biotinylated anti-mouse Igs (ZYMED Laboratories) followed by incubation with alkaline phosphatase-conjugated streptavidin (ZYMED Laboratories).

2.6. *Inhibition of the lipid transfer activity by anti-Id*

The transfer of PC from liposomes to mitochondria was examined according to the method of Bloj and Zilversmit [13]. Briefly, liposomes composed of egg yolk phosphatidylcholine and bis[hexadecanoyl]phosphate with a trace amount of 1-acyl-2-[$^{14}$C]linoleoyl-glycerophosphocholine and glycerol tri[9,10($n$)-$^{3}$H]oleate as a 'non-exchangeable marker' were used for the assay. Mitochondria were prepared from male albino rats (350 g) of the Wistar strain. A given amount of liposomes (12.5 nmol phospholipid phosphorus) was incubated with mitochondria (250 μg as protein) in the presence or absence of the partially purified PC-specific lipid transfer protein in 0.5 ml of 50 mM Tris/HCl buffer pH 7.4 containing 0.25 M sucrose and 1 mM EDTA. After incubation for 50 min at 37°C, the mitochondria were precipitated by centrifugation at 15 000 × $g$ for 15 min at 4°C, and the supernatants were counted in a Packard liquid scintillation counter (model 3320). The percentage transfer of 1-acyl-2-[$^{14}$C]linoleoyl-glycerophosphocholine from liposome to mitochondria was calculated from the formula as described previously [14]. In the inhibition assay, the PC-specific transfer protein was first mixed with various amounts of anti-Id or IgG from the pre-immune serum, and then liposome (12.5 nmol as phospholipid phosphorus) and mitochondria (250 μg as protein) were added to the mixture. The resulting mixtures were incubated for 50 min at 37°C and the percentage transfer of PC was determined as described above.

## 3. RESULTS AND DISCUSSIONS

### 3.1. *Specificity of anti-Id*

Polyclonal anti-Id was produced by immunizing rabbits with PC-specific mAb, JE-1. Among 3 rabbits immunized with JE-1, one rabbit with the highest anti-Id titer was selected and anti-Id was prepared from the serum. The specificity of the anti-Id was examined by ELISA. The anti-Id bound strongly to JE-1 and to another PC-specific mAb, JE-8, but not to anti-PS mAbs (PS4A7 and PS3A), nor to anti-phosphorylcholine mAb TEPC 15 (Fig. 1). No appreciable binding of the IgG purified from the pre-immune serum was observed in any of the mAbs examined (data not shown). The reactivities of mAbs that recognize phosphorycholine have been extensively studied [15–17]. Although these mAbs have been shown to recognize a negatively charged phosphate and the trimethyl structure of the choline residue [17], our previous analysis [7] indicated that the anti-PC mAbs had reactivity profiles distinct from those of anti-phosphorylcholine mAbs. The fact that the anti-Id raised in this study could not cross-react with the anti-phosphorylcholine mAb (TEPC 15) indicates that the anti-PC mAbs may belong to a distinct family of the anti-phospholipid antibodies and the anti-Id may recognize some unique structure expressed on the anti-PC mAbs. To determine whether the anti-Id reacted with the combining site related idiotopes, the inhibition of the binding of anti-phospholipid antibodies to phospholipid antigens was examined. The anti-Id totally inhibited the binding of JE-1 to PC and partially inhibited the binding of JE-8. The anti-Id had no effect on the binding of other mAbs (PS4A7 and PS3A) (Fig.

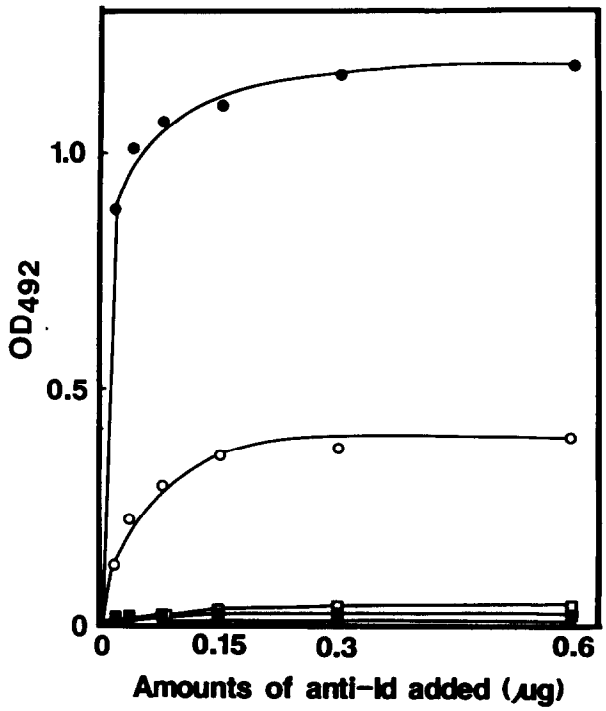


Fig. 1. Detection of idiotypic determinants on anti-phospholipid mAb. Microtiter plates were coated with anti-PC (•, JE-1: ○, JE-8), anti-PS (□, PS4A7: ■, PS3A) mAbs and TEPC 15 (▲). The anti-Id bound was detected with biotinylated anti-rabbit IgG and peroxidase-conjugated streptavidin.

2). The result suggests that the anti-Id recognizes idiotypes that are identical or spatially in proximity to the combining sites of the anti-PC mAbs.

### 3.2. *The anti-Id cross-reacts with PC-specific lipid transfer protein*

Since the reactivity profile of JE-1 showed a close similarity to that of the PC-specific lipid transfer protein [7,8], the cross-reactivity of the anti-Id with the PC-specific lipid transfer protein purified from bovine liver was examined. As shown in Fig. 3, the anti-Id did bind to the transfer protein, but the control IgG from the pre-immune serum did not (Fig. 3). The binding of the anti-Id to the PC-specific lipid transfer protein was inhibited by both JE-1 and JE-8, but not by anti-PS mAb, PS4A7 (Fig. 4). This observation further confirms that the anti-Id recognizes the cross-reactive determinants expressed on both the anti-PC mAbs and the PC-specific lipid transfer protein. Finally, we examined whether the anti-Id inhibits the lipid transfer activity of the PC-specific lipid transfer protein (Fig. 5). The partially purified PC-specific lipid transfer protein (about 3 μg) which is capable of transferring 25% of phosphatidylcholine molecules between the vesicles and mitochondria in the present assay system was selected for the inhibition analysis. The anti-Id inhibited the transfer activity of the PC-specific lipid transfer protein in a dose dependent manner, whereas the IgG purified from pre-immune sera did not inhibit the transfer. Recent findings showed that anti-Id could recognize cross-reactive structures shared by the antibody and the receptor molecules, and some of the anti-Id carry the internal image properties of the ligand molecule [6,18–20]. In these studies extremely high concentrations of anti-Id were required to compete with the

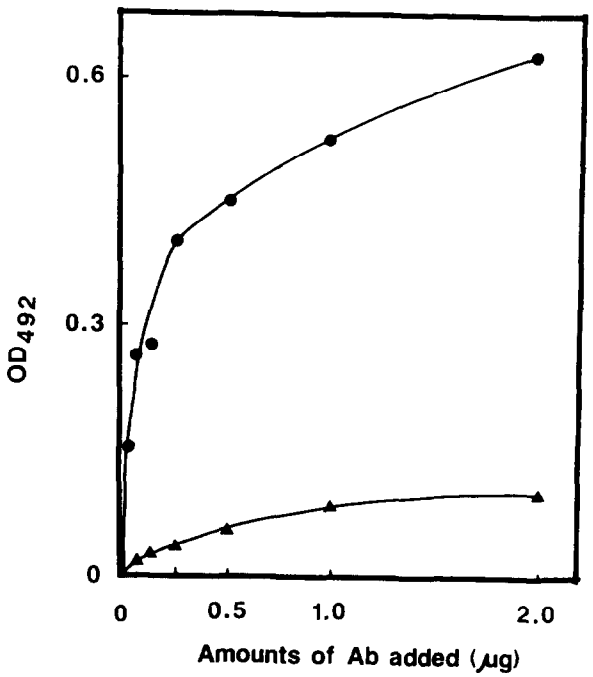


Fig. 3. Binding of anti-Id to the PC-specific lipid transfer protein. Microtiter plates were coated with the purified PC-specific lipid transfer protein and were incubated with various amounts of anti-Id IgG (•) or IgG from pre-immune serum (▲). The anti-Id bound was detected with biotinylated anti-rabbit IgG and peroxidase-conjugated streptavidin.

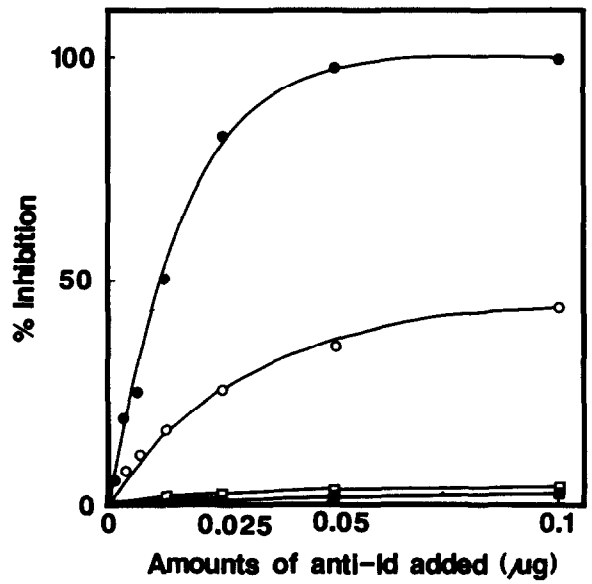


Fig. 2. Inhibition of the binding of the anti-PC mAbs by anti-Id. Anti-PC (•, JE-1: ○, JE-8) and anti-PS (□, PS4A7: (■), PS3A) mAbs were preincubated with various amounts of anti-Id, and the mixture was transferred to microtiter wells coated with the corresponding phospholipid antigens. The results are expressed as percentage inhibition of the binding measured in the absence of anti-Id.

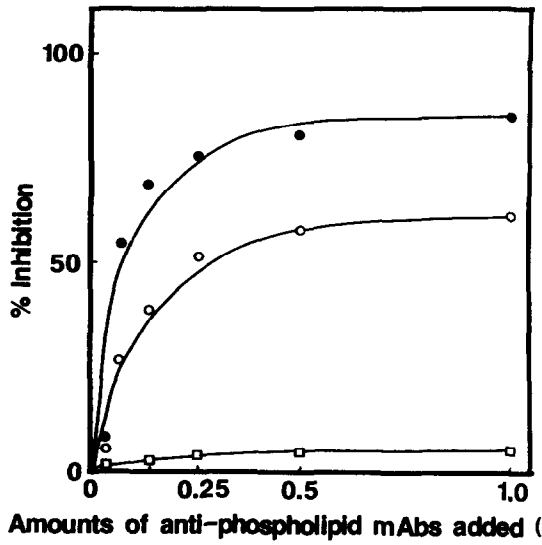


Fig. 4. Inhibition of the binding anti-Id to the PC-specific lipid transfer protein by anti-PC mAbs. Anti-Id was preincubated with various amounts of anti-PC (•, JE-1: ○, JE-8) and anti-PS (□, PS4A7) mAb. The mixtures were transferred to microtiter wells coated with the PC-specific lipid transfer protein. Anti-Id bound was detected by biotinylated anti-rabbit IgG and alkaline phosphatase-conjugated streptavidin.

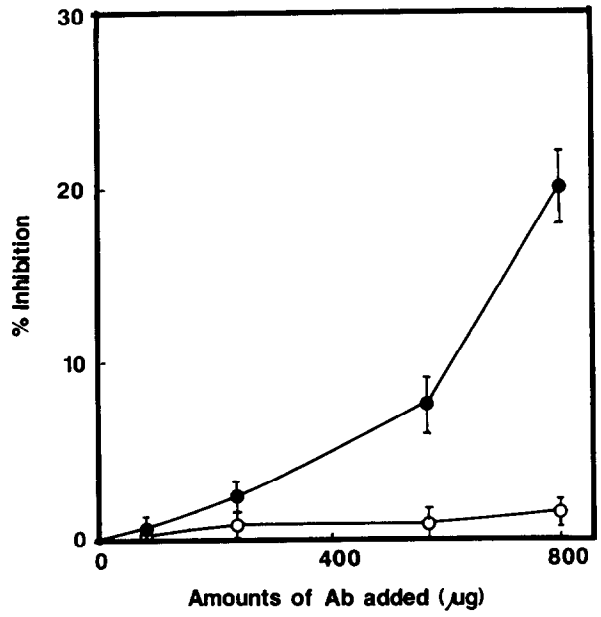


Fig. 5. Inhibition of the transfer activity of the PC-specific lipid transfer protein by anti-Id. Liposomes (12.5 nmol phospholipid phosphorus) and mitichondria (250 μg protein) were incubated with partially purified PC-specific lipid transfer protein in the presence of anti-Id IgG (•) or IgG from pre-immune serum (○). The results are expressed as percentage inhibition of the transfer activity measured in the absence of the antibody, and all values are the mean ± SD of 4 different experiments.

ligand molecules. In the present study the highest concentration of the anti-Id exhibited a mean of 23% inhibition of the transfer activity. Since we obtained extremely small amounts of IgG when the anti-Id was further purified by an affinity chromatography on JE-1 coupled to Sepharose (data not shown), the content of the anti-Id that carries the internal image properties of PC molecule may be too low to fully compete with the PC molecules for the binding to the PC-specific lipid transfer protein.

The present study revealed that the anti-PC mAbs and the PC-specific lipid transfer protein share the common structure that was identified by the anti-Id against anti-PC mAb, and some of the anti-Id may carry the internal image property of PC molecule. We hope that the anti-Id will provide useful tools for the identification and purification of a family of choline-containing glycerophospholipid binding proteins that have not been purified by the conventional procedures of the protein purification.

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