Characterization of a Novel Membrane Glycoprotein Involved in Platelet Activation*

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When platelets bind certain specific ligands they are induced to secrete the contents of their cytoplasmic granules and to aggregate. Studies of the molecular events accompanying this vital physiological response have led to a greater understanding of cell activation in general since the pathways involved are common to a number of cell types. By contrast most of the information about the cell surface molecules that initiate signal transduction has emerged from work on T lymphocyte activation, a process essential to the initiation of the immune response. We have described an activation antigen on T lymphocytes that is involved in the differentiation of these cells. In the present report it is demonstrated that the antigen is expressed on the platelet membrane with about 1,200 copies/platelet. A monoclonal antibody detecting this antigen stimulates platelet secretion and aggregation with a half-maximal response at ~10^{-8} M. Characterization of the antigen, termed PTAl, reveals a glycoprotein of M, 67,000 showing extensive N-linked carbohydrate, much of which appears to be heavily sialated. The amino-terminal sequence of PTAl, EEVLWHTSVPA-EXMSLEXYPSM, indicates that the protein has not previously been characterized. Preliminary investigation of the mechanism by which PTAl mediates platelet activation suggests involvement of protein kinase C and the 47-kDa protein of platelets is rapidly phosphorylated upon antibody-mediated activation. During this process PTAl is also phosphorylated, as it is following platelet activation by the other agonists, collagen, thrombin, and 12-O-tetradecanoylphorbol 13-acetate. These results provide the first example of a cell surface glycoprotein that is directly involved in both platelet and T lymphocyte activation.

Platelet aggregation and secretion in response to stimulation by specific agonists are central to the control of hemostasis, and the biochemical processes that accompany this dramatic activation event have been studied extensively. Results obtained with platelets have frequently provided insights of general significance to an understanding of cellular activation, and many of the intracellular pathways employed during activation appear to be shared by platelets and the great majority of nucleated cell types. Thus, studies on platelet aggregation were instrumental in elucidating the role of phospholipid turnover as a pathway of signal transduction (reviewed in Ref. 1) and in dissecting the role of Ca^{2+} and cyclic nucleotides in regulating this pathway (2, 3).

In platelets the initiating signal for activation is triggered by the binding of a specific agonist, such as collagen or thrombin, to its receptor on the platelet membrane. The molecular processes that cause triggering are not completely understood and further understanding of this process is being facilitated by structural studies of the specific receptors involved. The collagen receptor(s) on platelets, for example, is still open to question and more than one receptor may be involved in binding to this large molecule (4–11). Although it is possible that the various receptors bind to different types of collagen, detailed structural studies or these putative receptors have not resolved the process by which ligand binding initiates the activation of platelets, and there is no information on possible interactions between different potential receptors and signaling molecules within the same cell. Much more information on this aspect of cellular activation has come from the study of T lymphocytes. In these cells, activation can be triggered by antibodies that bind to the T cell receptor (12) or to the complex of proteins associated with this receptor, collectively termed CD3 (13–15). Antibodies to another T cell surface antigen, CD2, can also mediate activation (16), and an association exists between these two triggers of activation: for example, T cells lacking the CD3 antigen complex cannot be activated through CD2 (17), and, in addition, pretreatment of the lymphocytes with a monoclonal antibody to an epitope on CD2 that does not cause activation can deliver a negative signal that inhibits the signal delivered by antibodies to CD3 (18, 19). T cell activation through CD8 can also be inhibited or stimulated by different antibodies against another T cell antigen, the integrin (4) named LFA-1 (19). Hence it appears that quite distinct signaling molecules on the cell surface may be mediating their effects through a single activation system. Expression of CD2 and CD3 is restricted to T lymphocytes, but given the common pathways of secondary message transduction between platelets and lymphocytes (1, 19) it can be suggested that the initial transmembrane triggering events will also share a common

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mechanism. The discovery of a shared cell surface protein on both T lymphocytes and platelets that was involved in the activation of both cell types would be of potential importance toward elucidating the molecular basis of the primary events in activation and possible interactions between different receptors.

In the present report we describe the preliminary characterization of a cell surface antigen on platelets and demonstrate its involvement in platelet activation. The antigen is detected with a monoclonal antibody, LeOAl, that was initially isolated for its ability to bind an activation antigen on the surface of T lymphocytes and to inhibit the differentiation of these cells during mixed lymphocyte culture (21). In subsequent experiments we have found that the antibody can inhibit the differentiation of T cell clones in response to interferon (22) and, like anti-CD2, can inhibit the activation of T cells induced by anti-CD3.1 The antigen, termed PTA1, is the same M, (67,000) on platelets as that found on T cells (21), and a rabbit antiserum raised against purified platelet PTA1 inhibited T cell differentiation in mixed lymphocyte culture (25). Our studies reported here with the monoclonal antibody LeOAl show that the pattern of aggregation induced by LeOAl antibody is quite characteristic and different from that induced by other known platelet agonists, hence further study of this surface glycoprotein promises insights into possible novel pathways of cellular activation.

**EXPERIMENTAL PROCEDURES**

**Preparation of Human Platelets**—Unless stated otherwise, platelets were obtained as concentrates from the Red Cross Blood Center. Prior to use they were pelleted by centrifugation at 2000 x g for 15 min and washed twice in HEPES/saline/EDTA (10 mM HEPES, pH 7.4, 140 mM NaCl, 1 mM EDTA).

**Preparation of LeOAl Monoclonal Antibody**—LeOAl IgGl antibody was precipitated from ascites fluid by dropwise addition of saturated ammonium sulfate to 40% and dialyzed against phosphate-buffered saline, pH 7.2. IgGl was purified from this preparation by standard HPLC ion-exchange techniques, using a Bi-Gel TSK DEAE-5PW column.

**Competitive Binding Assay**—HPLC-purified LeOAl was labeled with Na refresh (Amersham Corp.) to a specific activity of 10 µCi/µg protein as described previously (21). 1 x 10^6 platelets were incubated with serial dilutions of radiolabeled LeOAl in a total volume of 100 µl of RPMI 1640 medium containing 1% (w/v) bovine serum albumin, 20 mM HEPES, pH 7.2, for 30 min at 4°C. Free and cell-bound [125I]-LeOAl were then separated by centrifugation through 80% silicone oil, 20% paraffin oil. The tips of tubes containing the cell pellets were removed and their associated radioactivity quantitated by counter.

**Platelet Aggregation and Inhibition Studies**—Citrated platelet-rich plasma (25), adjusted to give a final platelet concentration of 2 x 10^9/ml after all other additions, was incubated at 37°C. Luciferin-luciferase reagent (Chrono Log Corporation, Haverton, PA) and LeOAl (0.5-50 µg/ml final concentrations) were then added and the samples stirred at 900 rpm in a Payton 1000 dual-channel lumiaggregometer (Scarborough, Ontario). The aggregation of platelets was assessed by a change in transmittance of light through the suspension, as detected in one channel of the lumiaggregometer. The secretion of ATP from platelet granules was assessed by the change in luminescence of the protein as described previously (21).

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Polyacrylamide gel electrophoresis (27).

Phosphorylation of PTAl Antigen and Other Platelet Proteins—Platelets were prepared and resuspended in HEPES/saline/EDTA as described above to a concentration of 2 × 10⁶ platelets/ml. [³²P]orthophosphate (BRESATEC, Adelaide University, Australia) was used to label platelets at 500 μCi/ml for 90 min at room temperature. Platelets were then centrifuged and resuspended in normal Tyrode’s solution (138 mM NaCl, 2.9 mM KCl, 0.5 mM MgCl₂, 12 mM NaHCO₃, 0.3 mM NaH₂PO₄, 10 mM HEPES, pH 7.4, 5.5 mM glucose). Equal numbers of platelets were warmed to 37 °C for 5 min in this medium before the addition of 12-0-tetradecanoylphorbol 13-acetate (TPA) (50 ng/ml), thrombin (1 unit/ml), collagen (15 μg/ml), or LeoAl monoclonal antibody (5 μg/ml). After various time intervals cold phosphate-buffered saline was added to the platelets, which were immediately pelleted by centrifugation at 4 °C. Platelets were lysed in 1 ml of Nonidet P-40 (lysis buffer described above) containing the phosphatase inhibitors NaF (50 mM) and sodium orthovanadate (0.4 mM) (32). The samples were then analyzed directly or PTAl protein was immunoprecipitated from the platelet lysate as described above. (Where platelets were stimulated with soluble LeoAl antibody a 1:1 mixture of LeoAl-Sepharose CL-4B and rabbit anti-mouse Ig-Sepharose CL-4B was used for immunoprecipitations.) Samples were analyzed by one-dimensional and two-dimensional gel electrophoresis.

RESULTS

The PTAl Antigen Is Involved in Platelet Activation—PTAl antigen was first described as an early activation antigen on human T cells (21) identified by a monoclonal antibody termed LeoAl. A subsequent survey of the tissue distribution of PTAl antigen by alkaline phosphatase anti-alkaline phosphatase staining unexpectedly revealed that this antigen was also present on the surface of both blood platelets and platelet precursors (megakaryocytes) in the bone marrow (not shown). The antigen was originally described as T lineage-restricted activation antigen-1 to reflect its lineage restriction but because of its important role on platelets we have renamed the protein PTAl.

The antibody LeoAl was tested to determine its effect on platelet function. The antibody was found to be a potent stimulator of both platelet aggregation (see below) and release (Fig. 1a). The concentration of LeoAl required for half-maximal response as measured by release of dense body ATP was 1.5 μg/ml (~10⁻⁹ M) (Fig. 1a). A similar dose-response curve was observed for platelet aggregation (Fig. 1). Interestingly, increasing concentrations of antibody did not increase the amplitude of the aggregation trace (the percent aggregation) but rather the time before onset of the response (Fig. 1). Thus, as shown in Fig. 2 with washed platelets, the period of time before the onset of both shape change and aggregation was directly related to the concentration of antibody used, varying between 10 s at 25 μg/ml to 60 s at 0.4 μg/ml, and indeed the time before aggregation could be as long as 5 min at very low concentrations of LeoAl (not shown). Similar dose-response data were reproduced in five subjects, and the aggregation by LeoAl at 50 μg/ml was confirmed in 14 donors. That the response observed was indeed aggregation rather than antibody-mediated agglutination was confirmed in two ways. Platelets from a patient with Glandmann’s thrombasthenia were tested, and while these platelets exhibited LeoAl-induced granule release (not shown) and shape change they did not aggregate (Fig. 1f). In addition, the aggregation and release induced from normal platelets by LeoAl were totally abrogated in the presence of a monoclonal anti-GPIIb/IIIa antibody (see below).

3 Figs. 1a–1c are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

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FIG. 1. Platelet aggregation induced by binding of LeoAl antibody. The aggregation traces show the response of citrated platelet-rich plasma to increasing concentrations of LeoAl: trace A, 0.5 ng/ml; trace B, 1 ng/ml; trace C, 2 ng/ml; trace D, 5 ng/ml; trace E, 50 ng/ml. Aggregation trace F shows the shape change without aggregation induced by 0.5 μg/ml LeoAl when added to citrated platelet-rich plasma from a patient with Glandmann’s thrombasthenia.

FIG. 2. Scatchard analysis of [²⁵I]-LeoAl binding sites on human platelets. 1 × 10⁶ platelets were incubated with [²⁵I]-LeoAl antibody for 60 min at 4 °C before separation of the bound and free counts/min. Nonspecific binding was determined by incubation of parallel tubes with an excess of LeoAl antibody. The data were evaluated by Scatchard analysis.

There have been reports previously describing monoclonal antibodies to two major platelet proteins (CD9 and GPIIb-IIIa) that activated and aggregated platelets (33–35), but these antigens were present in high copy number on the platelet surface (40,000–50,000). In order to determine the number of A1 molecules present on human platelets a competitive binding assay was carried out with LeoAl. In the representative binding study shown in Fig. 2 the antibody bound with a Kᵣ of 1.2 × 10⁻⁹ M, and Scatchard plot analysis revealed there were 1,200 LeoAl binding sites/platelet. Similar data were obtained with platelets from three different subjects.

Biochemical Characterization of Platelet PTAl—The low copy number for PTAl suggested a significant role for this antigen in platelet activation. It was therefore of interest to investigate the biochemical characteristics of this surface molecule. PTAl protein was purified from platelet concentrates by affinity chromatography. An aliquot of the purified protein was analyzed by one-dimensional SDS-PAGE coupled with silver staining which revealed a diffuse glycoprotein band of 67–70 kDa (Fig. 3) under reducing conditions. The same size molecule was immunoprecipitated with LeoAl antibody

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from surface iodinated platelets and when analyzed under nonreducing conditions (Fig. 3) PTA1 antigen showed a small apparent increase in mobility, perhaps suggesting the presence of intramolecular sulfydryl bonding. The diffuse appearance of the protein on SDS-PAGE suggested extensive glycosylation. To test this, N-linked carbohydrate was removed by the incubation of immunoprecipitated PTA1 from surface iodinated platelets with Endo F and the same enzyme reduced the apparent molecular weight of PTA1 to approximately 35 kDa, although the band seen after Endo F treatment is still relatively diffuse. However, the result was obtained on three occasions with different batches of Endo F and the same enzyme reduced the apparent molecular weight of platelet GPIIb-IIIa to the published size. Native platelet PTA1 antigen therefore is heavily glycosylated, and as the molecular mass of a single N-linked sugar is generally the order of 3–4 kDa it is possible that PTA1 on platelets possesses up to 10 N-linked carbohydrate sidechains. Immunoprecipitated protein from surface iodinated platelets was also analyzed by O’Farrell two-dimensional gel electrophoresis. PTA1 antigen had a pI of 3.5–4.2 (Fig. 4A). When sialic acid residues were removed by treating immunoprecipitated PTA1 with neuraminidase, the pI value underwent a marked shift to 7.8–8.2 (Fig. 4B), indicating the presence of a large number of sialic acid groups on the native antigen.

To obtain information about the primary structure, purified and carboxymethylated PTA1 was used to obtain NH2-terminal sequence data (Fig. 3). From 1011 platelets, 10 μg (286 pmol) of PTA1 antigen suitable for sequencing was obtained. The sequence shown in Fig. 3s was compared with protein sequences recorded in the National Biomedical Research Foundation (NBRF) Protein Data Bank using Beckman’s Microgenie sequence software and was found to represent unique NH2-terminal sequence. The high percentage of polar amino acids in the sequence indicates that the amino terminus of PTA1 is hydrophilic.

Investigation of the Mechanism of A1-mediated Platelet Activation—Inhibitors specific for individual pathways of platelet activation can provide useful information about the particular mechanism by which an agonist stimulates platelets. LeoA1-induced platelet aggregation and secretion (Fig. 5A) were essentially unaffected by either inhibitors of cyclooxygenase (indomethacin; Fig. 5B) or by scavengers of released ADP (creatine phosphate/creatine phosphokinase; Fig. 5C). The combination of indomethacin and creatine phosphate/creatine phosphokinase had a partial inhibitory effect; however, both aggregation and secretion still occurred after a longer lag period (data not shown). These data indicate that LeoA1 does not exclusively activate platelets through either a cyclooxygenase-dependent pathway and/or by the release of dense body ADP but, like strong agonists such as high dose collagen and α-thrombin (36), can cause platelet secretion and aggregation by another pathway of activation, possibly involving protein kinase C (37). Indeed, in a similar manner to physiological platelet agonists such as α-thrombin and collagen, LeoA1-induced platelet aggregation and secretion were strongly inhibited by 5 μM prostaglandin E1 (Fig. 5D) and by 30 μM forskolin or dibutyryl-cyclic AMP (1 μM) (not shown), which elevate cytoplasmic levels of cyclic AMP and thereby inhibit the activation of protein kinase C (38).
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**FIG. 6.** The protein kinase C inhibitor, H7, inhibits LeoA1-induced platelet aggregation. Washed platelets were incubated with H7 at the given concentrations for 2 min prior to the addition of agonist. Where H7 was not added, an equal volume of resuspension buffer was added. In A is shown the relationship between the concentration of H7 and the lag time before the onset of aggregation (measured as in Fig. 3). B shows the effect of increasing concentrations of H7 on the percentage aggregation. The percent aggregation is calculated from the height of the aggregation curve in the presence of platelets divided by the height of the light transmission trace in the absence of platelets (maximal light transmission). Error bars at nil H7 concentrations represent the standard error for four aggregations in the absence of inhibitor. Triangles correspond to aggregation induced with LeoA1 at 1 µg/ml, circles correspond to aggregation induced by 0.25 unit/ml thrombin.

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**FIG. 7.** Phosphorylation of platelet proteins induced by LeoA1 and other platelet agonists. Platelets were labeled for 90 min at room temperature using [32P]phosphate and then divided into equal aliquots and warmed to 37 °C. Platelets were either untreated (lane 5) or stimulated for 30 s with 5 µg/ml LeoA1 (lane 1), 50 ng/ml TPA (lane 2), 1 unit/ml thrombin (lane 3), or 12 µg/ml collagen (lane 4). Samples were then immediately pelleted and lysed and analyzed by SDS-PAGE.

5E also serves to illustrate that a monoclonal antibody to the platelet aggregation/fibrinogen receptor GPIIb-IIIa also inhibited aggregation, indicating that the aggregation event occurred through this receptor.

Further evidence possibly implicating protein kinase C involvement was obtained with H7, a potent inhibitor of protein kinase C (39). The presence of this inhibitor abrogated LeoA1-induced aggregation of washed platelets at between 400 and 500 µM for different donors, and over a range of lower concentrations H7 both increased the lag time before the onset of shape change and aggregation and reduced the amplitude of the aggregation curve (Fig. 6). Another parameter of platelet activation in response to physiological agonists is the increased phosphorylation of an intracellular protein termed p47. As shown in Fig. 7 it is apparent that both LeoA1 and TPA preferentially stimulated the phosphorylation of a 47-kDa protein.

Of interest was the finding that the PTA1 protein itself showed increased phosphorylation in response to LeoA1 binding to intact platelets, as revealed by immunoprecipitation from stimulated [32P]phosphate-loaded platelets (Fig. 8). In some experiments the PTA1 protein immunoprecipitated from 32P-loaded platelets appeared to be endogenously phosphorylated (Fig. 8A), but this was not a consistent observation (Fig. 8, B and D) and possibly reflects some platelet activation having occurred during cell preparation. Increased PTA1 phosphorylation was consistently observed in response to LeoA1-induced activation, but activation with other agonists including thrombin, collagen, and TPA caused increased PTA1 phosphorylation in some experiments but not in others (see e.g. Fig. 8, A and B); the reason for the different results obtained is not known. Two-dimensional gel analysis of the PTA1 protein phosphorylated in response to TPA showed no marked shift in pI, indicating that it is the mature glycosylated form that was being phosphorylated (Fig. 8C). The amino acids being phosphorylated have not been analyzed but the phosphorylation of PTA1 by activation induced by TPA further suggests the involvement of protein kinase C, and pretreatment of the platelets with H7 (400 µM) also markedly inhibited the phosphorylation of PTA1 induced by LeoA1 antibody (Fig. 8B). The phosphorylation of PTA1 was rapid, being observed within 10 s (Fig. 8D) and the protein remained phosphorylated when analyzed 15 min after platelet activation. The experiment illustrated in Fig. 8D was carried out with antibody at a concentration of 5 µg/ml, and comparison with the time course of shape change induced with the anti-
body (Fig. 2s) reveals a striking correlation between the time of maximal phosphorylation (Fig. 8D, track 2) and the initiation of shape change at this concentration. While not proving any direct linkage between the two events these data suggest that the phosphorylation of PTA1 may be physiologically relevant.

DISCUSSION

This study provides evidence for and preliminary characterization of the first cell surface glycoprotein to be directly involved in the activation of both platelets and of T lymphocytes. The protein is detected with a monoclonal antibody, LeoAl, and because of its involvement in platelet and T cell activation we have named the antigen platelet and T cell activation antigen 1 (PTA1). Binding of PTA1 by LeoAl directly induces platelet secretion and aggregation. This novel glycoprotein was partially characterized and its amino-terminal sequence shows no homology to other proteins described previously.

The rate of platelet aggregation and secretion induced by LeoAl was related to the concentration of antibody. Aggregation itself was mediated through GPIIb-IIIa as indicated in experiments using platelets from a patient with Glanzmann's thrombasthenia, a hereditary disorder caused by the absence of GPIIb-IIIa expression (26), and by inhibition of LeoAl-induced aggregation by an antibody to GPIIb-IIIa. These experiments also served to demonstrate that the LeoAl antibody was not simply agglutinating the platelets and revealed that the mechanism of aggregation being induced was similar to that caused by thrombin, collagen, and other agonists and distinct from the effects of ristocetin. The prolonged lag time before aggregation (although much less pronounced) is more reminiscent of collagen-induced activation than that induced by thrombin, and Rink et al. (2), using a fairly insensitive method, found that collagen activates protein kinase C in platelets without markedly raising the levels of intracytoplasmic Ca++. In this same study, the phorbol ester TPA had the same effect, and platelet secretion and aggregation in response to TPA also exhibited a lag period before onset; pretreatment of the platelets with low concentrations of calcium ionophore (subthreshold for secretion) induced an almost instantaneous response.
upon the addition of TPA (2). These results perhaps suggest that the amounts of Ca\(^{2+}\) released from the intracytoplasmic pool in response to stimulation by LeoA1 might be limiting. However, TPA, unlike LeoA1, does not induce any marked change in platelet shape (40–42), and we have found that pretreatment of the platelets with the calcium ionophore A23187 did not markedly accelerate aggregation induced with low concentrations of LeoA1.\(^4\) Nevertheless, evidence is provided that LeoA1-mediated platelet activation may involve the activation of a protein kinase C. Thus stimulation with LeoA1 or TPA both induced preferential phosphorylation of a 47-kDa platelet protein. While the function of this protein is not known its sequence has been deduced and contains a region resembling other protein kinase C phosphorylation sites (43). Also, although indirect, our studies with inhibitors would exclude involvement of most other known pathways leading to platelet aggregation. In particular, H\(^7\), although not entirely specific, is a potent inhibitor of protein kinase C (39) and this reagent prevented LeoA1-induced aggregation and phosphorylation of the PTAl antigen. It is of some interest that the inhibition caused by H\(^7\) was almost complete during LeoA1-induced aggregation, but much less so during thrombin-induced aggregation. It is possible that the inhibitory effect of H\(^7\) may vary with different isoenzymes of protein kinase C and that these data may suggest that activation mediated through PTAl is stimulating a restricted set of protein kinase C isoenzymes, possibly a Ca\(^{2+}\)-independent protein kinase C that is known to be present in platelets (44). The PTAl protein was purified from platelets by immune absorption to antibody-coated beads. This procedure yielded 10 \(\mu\)g of protein from 10\(^11\) platelets. Since we have shown by Scatchard analysis that there are only approximately 1000 LeoA1-binding sites/platelet, this figure would represent a very high yield of surface PTAl. However by immunoelectron microscopy we have found that there is a considerable intracytoplasmic pool of PTAl antigen localized, like GPLIIP-IIIa (45), in membrane-bound vacuolar structures and tortuous channels.\(^5\) Hence the immunopurified material presumably represents a mixture of surface and intracellular PTAl. Immunopurified PTAl obtained from platelets was shown to be a pure protein with a significant proportion of this being accounted for as N-linked polysaccharides. The acidic nature of the protein was also attributable to carbohydrate groups since the removal of sialic acid residues with neuraminidase altered the pi to approximately 8.0. Removal of N-linked carbohydrate revealed a core protein of around 35 kDa, although the additional presence of O-linked sugars cannot be excluded. Indeed, the diffuse character of the Endo F-treated protein (Fig. 3) suggests that PTAl does bear O-glycosylated sites or has N-glycosylation sites that are Endo F-resistant. Initially the purification of PTAl was difficult to accomplish and the yield was low, even though large numbers of platelets were utilized. However, the protein was detected in platelet lysates and in a standard platelet extract (25). While of some interest, these preliminary structural data do not provide sufficient information to indicate whether or not the PTAl protein is likely to function as a receptor for an, as yet, unknown ligand or is involved in signal transduction in general.

Nor is there sufficient structural detail to provide any insights into the mechanisms of signal transduction through PTAl. We have recently obtained cDNA clones coding for the T cell antigen\(^6\) which should be useful in this regard.

Involvement of the protein in the activation process was indicated in experiments showing that PTAl was phosphorylated rapidly (within 30 s) upon platelet stimulation with LeoA1 antibody. Very few membrane glycoproteins are characteristic of the antigen is possibly of significance and the specific amino acids that are phosphorylated are currently under investigation. Whatever the final outcome of these studies, we believe that the PTAl protein is the first platelet membrane protein to be implicated in what is possibly a novel pathway of activation which may be shared by T lymphocytes, and as such it provides a unique model for further study.

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