Receptor-independent Activation of Guanine Nucleotide-binding Regulatory Proteins by Terminal Complement Complexes

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Activation of heterotrimeric guanine nucleotide-binding proteins (G proteins) by terminal complement complexes (TCC) was investigated in human lymphoblastoid B-cell line JY25 and its mutant YJ5 deficient in glycosylphosphatidylinositol-anchored proteins. TCC assembly achieved by antibody-dependent activation of C7-deficient serum reconstituted with C7 increased specific guanosine-5'-(γ-thio)triphosphate (GTPγS) binding, 4- and 8-fold, in JY25 and YJ5 membranes, respectively, between 2 and 10 min, over the level without C7. TCC also increased GTPase activity 5- and 4-fold in JY25 and YJ5, respectively, between 5 and 10 min. Increased GTPase activity was noted first with C5b-7 assembly, which increased further with C5b-8 and C5b-9. The presence of G proteins in anti-TCC immunoprecipitates of cell lysates was investigated by demonstration of Ga subunit that can be ADP-ribosylated by pertussis toxin (PTX). Immunoprecipitated TCC complexes contained a PTX-sensitive 41-kDa Gia/Goa subunit, as shown by SDS-PAGE and Western blotting. These complexes were functionally active as determined by GTPγS binding. We have further shown that enhanced TCC elimination from the plasma membrane induced by TCC-generated signals was inhibited by PTX. In conclusion the biological activities induced by TCC in nucleated cells may be mediated in part by activation of PTX-sensitive G proteins.

Complement activation in infection and inflammation plays a crucial role in host defense by generating inflammatory mediators, such as C3a and C5a, by opsonization of activating particles through C4b, C3b, and iC3b and lysis of target cells by forming C5b-9 channels. Assembly of the cytolytic C5b-9 complex is accomplished through a sequential interaction of C5-C9 plasma proteins, which results in amphipathic conformational changes of C6-C9 and insertion of these peptides into the lipid bilayer. Assembly of C5b-9 channels occurs stepwise manner, by forming membrane-associated C5b-7, C5b-8, and C5b-9 complexes, collectively called as terminal complement complexes (TCC) (1-3). Unstable voltage-dependent pores are first detected when C8 interact with membrane-bound C5b-7 to form C5b-8. Binding of one C9 to a C5b-8 produces transmembrane channel of 1–3-nm pore size, which enlarges to 10 nm with increasing number of C9 and C9-C9 polymerization (3–5). When complement is activated on cells of homologous species, complement inhibitory proteins severely restrict the number and the pore size of the C5b-9 channel (6–9).

The C5b-7, C5b-8, and limited C5b-9 stimulate cells in the absence of lysis and induce a variety of biological activities, which include production of eicosanoids and their derivatives, generation of oxygen radicals, synthesis of tumor necrosis factor and interleukin-1, enhanced production of collagen and collagenases, hydrolysis of myelin basic protein, platelets activation, elimination of potentially lytic TCC, and generation of mitotic signals (1, 2, 10–13). These activities are mediated in part by increased [Ca2+]i and protein kinase C activity during C5b-8/C5b-9 pore formation (14–16). Increased mass levels of sn-1,2-diacylglycerol (DAG) and ceramide, known endogenous regulators of protein kinase C activity, were achieved by C5b-7, C5b-8, and C5b-9 in intact cells (17). Production of these signal messengers by C5b-7, which does not form a channel nor causes Ca2+ influx, may explain some of cellular activities induced by C5b-7 (11, 15, 17). The TCC-induced DAG increase was inhibited by prior treatment of cells with pertussis toxin (PTX), which suggested that activation of phospholipases by TCC may involve G proteins sensitive to PTX (17).

Heterotrimeric G proteins transduce signals from the receptor, and activate effector molecules, such as adenyl cyclases, phospholipases, and various ion channels (18–20). Ligand binding causes enhanced interaction of the receptor with corresponding Go and promotes rapid release of GDP from the α subunit. The activated receptor (R*) is associated with empty Ga (a1) with a high affinity together with β and γ subunits and stabilizes R*α_Gaγ complex. Subsequent entry of GTP to the binding site is thought to induce a transient decrease in affinity for both receptor and βγ subunits (19). Hydrolysis of GTP to GDP by intrinsic GTPase activity induces the interaction of α with the effector, then the α-GDP reassociates with the βγ. Interaction of α-GTP with effectors regulated by Mg2+ and Li+ is also affected by covalent modification of the α subunit, as in ADP-ribosylation induced by bacterial toxins (21, 22). G proteins are mostly activated by ligand-receptor interaction, and these receptors belong to proteins with seven membrane-spanning domains or a single membrane-spanning domain with or without intrinsic tyrosine kinase activity (20, 23, 24). G proteins can also be activated in receptor-independent manner by complexing with metallofluorides or by mastoparan. [AlF4]- or [BeF4]- activates G proteins by interacting with the nucleotide-binding site of α subunit, mimicking the γ-phosphate of GTP
This study presents experimental evidence that all of the three TCC complexes are capable of activating PTX-sensitive G proteins in the membrane. Physical association of TCC with the 41-kDa Ga subunit was demonstrated by immunoprecipitation with antibodies to C7, C8, and to C5b-9 neoantigen from detergent-solubilized cells carrying TCC. Involvement of G proteins in biological activities of TCC was suggested by the ability of PTX to inhibit the enhanced TCC elimination from the cell surface.

**EXPERIMENTAL PROCEDURE**

**Chemicals and Reagents—Cholera toxin (CTX) and PTX were obtained from List Biological Laboratories (Campbell, CA). Diithiothreitol (DTT), EDTA, EGTA, Lubrol, ATP, NAD, creatine phosphokinase, creatine phosphate, dextran (2 x 10^5), Norit A charcoal, bovine serum albumin (BSA), aprolin, leupeptin, and phenylmethylsulfonyl fluoride (PMSF) were from Sigma. GTPyS, GTP, GDP, AppNHp, and Triton X-100 were from Boehringer Mannheim. [γ-32P]GTPyS (3000 Ci/mmol), [35S]GTPyS (1267 Ci/mmol), and [32P]NAD (30 Ci/mmol) were from Du Pont NEN. Nickolase membranes were from Maryland Bedford, MA; BSA 0.85% nickel membrane filters (0.45 µm) were from Schleicher & Schuell; polyacrylamide reagents were from Bio-Rad; protein A-agarose and BCA reagent were from Pierce Chemical Co.

**Antibodies, Complement, and Complement Components—Mouse IgG monoclonal antibody to class II was precipitated with rabbit complement. The culture supernatant of hybridoma L-243 (ATCC, HB55, Batch P-8310) with used as source of complement components was obtained by pooling sera than JY25 (17, 31, 32). The antibody optimal for sublytic complement C9, and monoclonal IgG to human C5b-9 neoantigen (anti-C5b-9) were also obtained from Calbiochem-Novabiochem. Agarose-conjugated protein standards were also obtained from Calbiochem-Novabiochem. Rabbit antiserum to common Ga (19, 47) was purchased from Quidell (La Jolla, CA). Rabbit antiserum to common Ga subunit (GA1) and to G3a (EC2) were purchased from Du Pont NEN. Affinity-purified rabbit IgG specific for Glu, Gin, and to Gs subunit were purchased from Calbiochem-Novabiochem. All other antibodies were purchased from Sigma.

**Preparation of Cells and Cell Membranes—The JY cell line is an Epstein-Barr virus transformed human lymphoblastoid B-cell line. JY, a derivative of JY5, has a high percentage of expressing gsp mutants. Phosphatidylinositol-anchored membrane proteins which include diacyl-glycerol-phosphatidylinositol-specific and phospholipid-specific lectin binding (17, 31, 32). The antibody optimal for sublytic complement activation was determined by lysis of JY25 or JYS sensitized by serial dilutions of monoclonal IgG to HMC class II protein with excess serum complement (10% NHS or 20% C7D + 10 µg of C7, as described (17)). Identical concentrations of IgG and serum complement were used in experiments performed with plasma membranes. Plasma membranes were prepared according to a procedure described (33), with several modifications. In brief, 5 x 10^6 JY cells were stirred for 10 min in 100 ml of 20 mM borate buffer with 0.2 x EDTA, pH 10, and again for 5 min after addition of 10 ml of 500 mM borate buffer. Cells were filtered through 120-µm nylon mesh, and nuclei were removed by centrifuging for 5 min at 2000 rpm (Beckman AccuSpin FR) at 4 °C. Membranes were precipitated by centrifugation at 15,000 rpm for 30 min (Sorvall RC-5B), resuspended in PBS, and ultracentrifuged at 45,000 rpm for 60 min at 0 °C using SW60 rotor (Beckman Instruments Inc.). Membrane pellets were suspended in PBS containing protease inhibitors (1 mM PMSF, 10 µg/ml leupeptin, and 10 µg/ml aprotinin). Protein concentration was determined by using BCA reagent and membranes were kept aliquoted at −78 °C until use.

**Assay for GTPyS Binding and GTPase Activity—**For GTPyS binding, plasma membranes (50 µg of protein/sample) were incubated with 10 µg/ml anti-MHC class II IgG were suspended in 50 µl of Tris-buffered saline (TBS) (20 mM Tris-HCl, 100 mM NaCl, pH 8.0, containing 30 mM MgCl2, 1 mM EDTA, 1 mM DTT, 0.1% Lubrol, 1 µM GDP (34–36). Following addition of 10% NHS or 20% C7D + 10 µg of C7, and 0.1 ml of [35S]GTPyS (specific activity of 1250 Ci/mmol) diluted with TBS to 0.22 mM GTPy-S, the mixtures were shaken at 37 °C. The reaction was stopped at different time points by adding 950 µl of ice-cold TBS with 25 µM unlabeled GTPyS. Samples were vortexed and filtered through 0.45-µm BAAS filter. After washing two times with 10 ml of ice-cold TBS, filters were immersed in 10 ml of scintillation fluid, and the specific activity was measured. Membranes treated with NHS without IgG, or IgG and C7D without C7, were used as controls. Results, performed in duplicates or for five experiments for NHS and controls, were statistically analyzed by the paired Student's t test.

**GTPase activity was determined as described (34, 35). Membranes (10 µg of protein/sample) sensitized with 10 µg/ml IgG were incubated for 5 min at 37 °C with 10% NHS, 20% C9D, or C7D, then centrifuged at 37,000 rpm for 1 min in a Beckman Microfuge B. Membranes then incubated containing 300 µl of 10 mM MgCl2, 0.1 mM ATP, 3 mM creatine phosphokinase, 75 units/ml creatine kinase, 0.1 mM EDTA, 1 mM DTT, and 250 mM [32P]GTP (specific activity, 3000 Ci/mmol), then incubated at 37 °C in a shaker. Reaction was terminated at the indicated time points by adding 1 ml of cold 5% Norit A charcoal suspension in PBS containing 0.1% dextran and 0.5% BSA. Mixtures were centrifuged at 12,000 rpm for 1 min in a Beckman Microfuge, and the radioactivity in 200-µl supernatant was counted. Membranes treated with GTPase buffer without sera were used in each assay as additional control. Results of five experiments for NHS and three for deficient serum, performed in duplicates, were statistically analyzed by the paired Student's t test.

**ADP-ribosylation of JY Membrane Carrying TCC Complexes—**Untreated membranes and IgG-sensitized membranes (150 µg of membrane protein) were incubated with 10% NHS for 15 min at 37 °C. Membranes were washed and resuspended in 50 µl of ADP-ribosylation buffer (20 mM Tris-HCl, 50 µM thymidine, and 0.1 ml of TBS, 10 mM DTT, 1 mM EDTA) (36, 37) containing 10 µM [35S]NAD and 10 µg/ml PTX or CTX previously activated by incubating in 200 µM PTX or CTX at 10 °C. Membranes were then incubated in 1 ml of TBS with 1% Triton X-100 for 30 min on ice with repeated vortexing and sonication. Proteins in the membrane lysates were precipitated overnight at 4 °C with 20% trichloroacetic acid. Identical amounts of protein from each sample, determined by BCA, were boiled for 5 min in loading buffer (125 mM Tris-HCl, pH 6.8, 2% SDS, 5% mercaptoethanol, 40% sucrose, and 0.02% bromophenol blue) and analyzed 10% SDS-PAGE (33) followed by Western blotting and autoradiography. For immunodetection, blots were washed in TBS with 5% BSA and 0.1% Triton X-100 for 20 min at 25 °C, then exposed to antibodies to human Ga or Gb subunits. The antibody-reactive bands were visualized with horseradish peroxidase conjugated with goat IgG to rabbit IgG followed by the use of horseradish peroxidase-pre-precipitating reagent (Harlan, Madison, WI) or by enhanced chemiluminescence detection reagents (ECL) (Amersham Corp.). Autoradiographic densities were quantitated by Molecular Dynamic Densitometer, and the density of each band was integrated using Imagequant Software (Molecular Dynamics, Sunnyvale, CA). The results were expressed in arbitrary scale units.

**Immunoprecipitation of TCC-G Protein Complexes—**Intact cells (5 x 10^7) sensitized with IgG were incubated for 15 min at 37 °C with 10% NHS, 20% C9D or C7D, and also with 20% C7D ± 10 µg of C7. Cells were washed with Hanks' balanced salt solution, then placed in 1 ml of cold lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 10 µg/ml leupeptin, 10 µg/ml aprotinin, and 1 mM PMSF) for 30 min on ice, while vortexing and sonicating repeatedly. Cell debris were removed by centrifuging 5 min at 2000 rpm at 4 °C (Beckman AccuSpin FR). The supernatants were preincubated with protein A-agarose for 15 min at 4 °C and centrifuging at 15,000 rpm for 1 min in an Eppendorf centrifuge 5415 C. This detergent-solubilized cell lysates (100 µl) were incubated with 50 µg/ml anti-C5b-9, anti-C8, anti-C7, or anti-Ga IgG and with 100 µg/ml of agarose conjugated with secondary antibodies for overnight at 4 °C. Agarose beads were then washed four times with lysis buffer, twice with TBS, then subjected to ADP-ribosylation with preactivated PTX or CTX as described for the plasma membrane. The reaction was terminated by adding 1 ml of cold TBS. After washing, beads suspended in loading buffer were boiled for 3 min, and identical amounts of protein from each sample, determined by protein assay, were analyzed by 10% SDS-PAGE and autoradiography.

**TCC-G protein complexes isolated on agarose were also examined for the [35S]GTPyS binding. Ten µl of agarose carrying immunoprecipitated complexes, with and without 30-min exposure at 37 °C to 10 µg of CTX or PTX, were suspended in 50 µl of 150 mM NaCl, pH 8.0 containing 1 µl of [35S]GTPyS (12 µCi/sample). The mixtures were incubated at 37 °C in a shaker for 15 min. The reaction was terminated by adding 1 ml of ice-cold TBS containing 1 µM GDP, then...
the suspension was filtered through a 0.45-μm BA85 nitrocellulose filter. Filters were washed twice with 10 ml of cold TBS, and the radioactivity associated with the filter was counted. The data are mean ± S.E. from five experiments performed in duplicates.

**RESULTS**

Activation of Serum Complement and TCC Assembly Stimulate [35S]GTP-γS Binding and [γ-32P]GTP Hydrolysis—Possible activation of G proteins by serum complement was investigated by detecting nonhydrolyzable analogue [35S]GTP-γS binding to JY plasma membranes and also by GTPase activity. The increase in GTP-γS binding after addition of NHS to IgG-sensitized membranes between 2 and 10 min was 4- and 10-fold in JY25 (p < 0.02) and JY5 (p < 0.05), respectively, over the level by NHS in the absence of IgG (Fig. 1A). Hydrolysis of GTP, determined as GTPase activity, increased 13-fold between 1 and 15 min in JY25 (p < 0.05), whereas 4-fold increase between 1 and 10 min was the maximum in JY5 (p < 0.05), over the level with NHS alone (Fig. 1B). When membranes were pretreated with PTX (500 ng/ml), the extent of GTP hydrolysis induced by IgG and NHS was reduced to 50% (Fig. 2). Inhibition was also observed when the reaction was carried out in the presence of 10-fold excess GDP (data not shown). Incomplete inhibition of GTP hydrolysis by PTX may be in part due to the presence of PTX-insensitive G proteins.

To evaluate the role of TCC, GTP-γS binding and GTP hydrolysis were determined in IgG-sensitized membranes treated with C7D and C7. As shown in Fig. 3, C7 reconstitution was required to achieve the effect of IgG and NHS. Between a 2- and 10-min period, GTP-γS binding increased 4-fold in JY25 (p < 0.02) and 8-fold in JY5 (p < 0.05) over the level obtained by C7D without C7 (Fig. 3A).

The increase in GTP hydrolysis in response to C7 reconstitution between 5 and 15 min was 5-fold in JY25 (p < 0.02) and 4-fold in JY5 (p < 0.02) (Fig. 3B). When GTP hydrolysis was determined during assembly of C5b6, C5b-7, and C5b-8, using C7D, C8D, and C9D, the increase between 1 and 10 min was 2and 3-fold for C5b-7 and C5b-8, respectively, over the level by C5b6 (Fig. 4). Treatment with C7D + C7 used as maximum C5b-9 effect showed 5-fold increase in GTP hydrolysis between 1 and 10 min when compared with the level by C7D, which generates C5b6. C5b-9 is a strong activator as evidenced by a 2-fold increase in GTP hydrolysis at 1 min over the C5b6 level, which was similar to the basal level obtained without serum.

ADP-ribosylation of G Proteins by PTX in Membranes Carrying Inserted TCC—PTX-induced ADP-ribosylation of a protein migrated as a 41-kDa band which was two times more intense in JY25 membranes than in JY5 (Figs. 5 and 7). This band was identified as Gis/Goo by immunoreacting with specific antibodies to Gis/Goo subunits. When membranes previously treated with IgG and NHS were ADP-ribosylated by PTX, the reaction was significantly reduced (Fig. 5). These findings

![FIG. 1. GTP-γS binding and GTPase activity during complement activation](image)

A. plasma membranes (50 μg of protein) of JY25 (○), JY5 (□) and JY5 (○) sensitized with (circles) or without (squares) anti-MHC class II IgG (10 μg/ml) at 37°C for 30 min, were suspended in 50 μl of TBS buffer containing 30 mM MgCl₂, 1 mM EDTA, 1 mM DTT, 0.1% Lubrol, 1 μM GDP. Following addition of 10% NHS and 0.22 nm [35S]GTP-γS (1267 Ci/mmol) and incubation at 37°C, 950 ul of ice-cold TBS was added at the time indicated. The samples were filtered, and the radioactivity associated with the filter was counted. The data are mean ± S.E. from seven experiments performed in duplicates. B. JY25 IgG-sensitized membranes (10 μg of protein) were incubated with 10% NHS for 5 min at 37°C. Pelleted membranes were incubated in 50 μl of TBS containing 30 mM MgCl₂, 0.5 mM ATP, 3 mM creatine phosphate, 75 units/ml creatine phosphokinase, 0.1 mM EGTA, 1 mM DTT, and 250 nm [γ-32P]GTP (3000 Ci/mmol). At the time indicated, 1 ml of cold PBS containing 5% Norit A charcoal, 0.1% dextran, and 0.5% BSA was added, and radioactivity of 200-μl supernatant was counted. Data are mean ± S.E. from five experiments performed in duplicates.

![FIG. 2. Inhibition of GTPase activity by PTX. JY25 (●) and JY5 (□) membranes treated with preactivated PTX (500 ng/ml) for 3 h (PTX) at 37°C were sensitized and incubated with 10% NHS for 5 min. The kinetics of GTP hydrolysis determined in membranes treated with PTX or with TBS instead of PTX (JY25 (●); JY5 (○)) were shown. The data represent mean ± S.E. from three experiments performed in duplicates.](image)
Fig. 3. GTPγS binding and GTPase activity during activation of terminal complement proteins. A, GTPγS binding to JY25 (○, ■) and JY5 (□, □) membranes was determined as described in the legend to Fig. 1A, except that sensitized membranes were incubated with 20% C7D with (circles) or without (squares) 10 μg of C7. The data are mean ± S.E. from three experiments performed in duplicates. B, the time-dependent increase in GTPase activity was determined by measuring GTP hydrolysis in JY5 (○, □) and JY25 (■, □) membranes as described in the legend to Fig. 1B, except that 20% C7D was used with (circles) or without (squares) 10 μg of C7. The data are mean ± S.E. from three experiments performed in duplicates.

Fig. 4. GTPase activity at various steps of TCC formation. IgG-sensitized JY25 membranes (10 μg of protein) were incubated for 5 min at 37 °C with 20% complement deficient serum. C7D, C8D, and C9D were used to form C5b6, C5b-7, and C5b-8, respectively. C5b-9 complexes were formed by C7D + C7. GTP hydrolysis was determined at 1 and 10 min, as described in the legend to Fig. 1B.

Fig. 5. ADP-ribosylation by PTX of membranes carrying inserted TCC. A, untreated (JY25, JY5) and IgG-sensitized membranes (150 μg of protein) treated with 10% NHS (JY25/TCC, JY5/TCC) were incubated in 50 μl of ADP-ribosylation buffer (see "Experimental Procedures") with 10 μM iNAD (30 μCi/mmol) and activated PTX (10 μg/ml) for 30 min at 37 °C. Membranes were solubilized in 1 ml of TBS with 1% Triton X-100, 1 mM PMSF, 10 μg/ml leupeptin, and 10 μg/ml aprotinin for 30 min at 0 °C. Proteins were precipitated with 20% trichloroacetic acid and analyzed by 10% SDS-PAGE, Western blot, and autoradiography. ADP-ribosylated proteins by PTX migrated as a 41-kDa band. The same blot was then incubated with anti-Go rabbit antiserum (GA/1) followed by horseradish peroxidase-conjugated goat IgG to rabbit IgG and developed with horseradish peroxidase precipitating reagent. B, densitometric quantitation of the autoradiography shown in A is expressed as relative densities. ■, untreated membranes; □, membranes treated with IgG and NHS.
with serum complement for 15 min at 37 °C. Cells were solubilized in lysis buffer (described in the legend to Fig. 4) containing additional 0.5% sodium deoxycholate. The lysates were immunoprecipitated with antibodies to TCC, and the complexes were recovered on agarose conjugated with secondary antibodies. Agarose beads were washed with lysis buffer and were then released from the beads by boiling and analyzed by 10% SDS-PAGE, Western blot, and autoradiography. The 41-kDa ADP-ribosylated proteins are shown. ADP-ribosylated proteins by PTX-mediated inhibition of cellular activities induced by TCC. The complexes were immunoadsorbed on agarose and subjected to ADP-ribosylation by PTX as described in the legend to Fig. 5, then analyzed by 10% SDS-PAGE, Western blot, and autoradiography. The 41-kDa ADP-ribosylated bands are shown in the upper panel. The same blot was reacted with rabbit anti-Gia1,2 IgG, then developed by ECL (lower panel). The immunoreactive band is a 41-kDa protein, as identified by ADP-ribosylation.

**Fig. 6.** Coprecipitation of Ga with TCC by anti-C5b-9 IgG, anti-C7, or anti-C8 IgG. IgG-sensitized JY25 cells (5 x 10⁶) were incubated with serum complement for 15 min at 37 °C. Cells were solubilized in lysis buffer (described in the legend to Fig. 5) containing additional 0.5% sodium deoxycholate. The lysates were immunoprecipitated with antibodies to TCC, and the complexes were recovered on agarose conjugated with secondary antibodies. Agarose beads were washed with lysis buffer and treated with activated PTX in ADP-ribosylation buffer. Proteins were then released from the beads by boiling and analyzed by 10% SDS-PAGE, Western blot, and autoradiography. A, cells treated with C7D + C7 or NHS were immunoprecipitated with anti-C5b-9 IgG, and cells treated with C8D or C9D were immunoprecipitated with anti-C7 or anti-C8, respectively, as described. The ADP-ribosylated proteins migrated as a 41-kDa band. B, controls used for the specificity of immunoprecipitation are shown. ADP-ribosylated proteins by PTX were not detected in C9D-treated cell lysates immunoprecipitated with isotype control IgG (C9D/agarose), NHS-treated cell lysates immunoprecipitated with anti-C3 IgG (NHS/anti-C3), or NHS-treated cell lysates immunoprecipitated with anti-C8 IgG (H-NHS/anti-C8). C, immunoprecipitates with anti-C5b-9 (NHS/anti-TCC) or anti-Gia1,2 contained similar ADP-ribosylated proteins induced by PTX. Untreated membranes and immunoprecipitation with anti-C3 or isotype IgG served as controls.

TCC, and these complexes were also associated with βγ subunits.

As shown in Fig. 9, complexes isolated by anti-C5b-9 antibody displayed constant increase in [35S]GTPγS binding between 5 and 15 min which was inhibited by the pretreatment with PTX. Therefore, G proteins precipitated with TCC were functionally active, and the GTP binding sites on Ga were available for GTPγS, even after they were eluted from the membrane.

**Involvement of G Protein in Biological Activities of TCC**—Functional implication of TCC-activated G proteins was evaluated by examining PTX-mediated inhibition of cellular activities induced by TCC. Cells have the capacity to survive limited C5b-9 attack by actively eliminating potentially lytic TCC from the cell surface by vesiculation of membrane fragments and by endocytosis (2, 14). This process is mediated by signals generated through TCC-membrane interaction (15, 17). As shown in...
were activated during the assembly of TCC, which include GTPyS binding and GTP hydrolysis. Preincubation of cell or membrane lysates with IgG-sensitized membranes allows complement activation up to C6 and generates inflammatory peptides, such as C3a and C5a, which bind GTPyS, which was blocked by PTX. The nature of molecular interaction between TCC peptides and G proteins is unknown. It is possible that TCC-G protein interaction may be similar to that of mastoparan, a tetradecapeptide toxin from wasp venom which activates heterotrimeric Gi, Gs, and Go in a receptor-independent manner by binding to the amphiphilic helix of Ga (27–29), as well as small G proteins from ras-related rho/rac family (39). Although, diverse G proteins sensitive to PTX or CTX were present in JY membranes, TCC peptides predominantly interacted with Giα/Goα species with a molecular size of 41 kDa in this cell line. Whether TCC would interact with G proteins with a similar restriction in other cell types remains to be determined.

Physiological significance of G protein activation during sublytic complement attack was evaluated by testing the TCC elimination from the cell surface by endocytosis and membrane shedding, processes known to depend on signals generated by TCC (2, 15, 17). Elimination of C5b-8 was completely inhibited by PTX, as shown by the functional assay. On the other hand, CTX, which failed to block the elimination, showed a slightly enhanced TCC effect. Interestingly, endocytosis or exocytosis are influenced by GTPyS, AIF/GTPγS, or mastoparan, also through a process involving activation of Giα/Goα (39–42). In addition, other biologically important inflammatory mediators, such as interleukin-8, tumor necrosis factor, FMLP (formylmethionyl-leucylphenylalanine), and C5a act on target cells through a receptor-independent mechanism.

Formation of sublytic TCC occurs during complement activation under diverse physiological conditions in vivo, as disparate as infection, ischemia, tissue necrosis, and neoplasia (47–50). Activation of target cells by TCC through involvement of Giα/Goα proteins in parallel with the increase in [Ca²⁺], may serve as bioregulatory mechanisms important in host defense by promoting cellular repair and tissue healing.

DISCUSSION

In this study we demonstrated that PTX-sensitive G proteins were activated during the assembly of TCC, which include C5b-7, C5b-8, and C5b-9 complexes. As shown in Figs. 1 and 2, antibody-dependent activation of serum complement induced GTPyS binding and GTP hydrolysis. As shown in Figs. 1 and 2, antibody-dependent activation of serum complement induced GTPyS binding and GTP hydrolysis. To assess the role of TCC, serum depleted of terminal complement proteins, C7, C8, or C9, was used to assemble various TCC complexes. The use of C7D on IgG-sensitized membranes allows complement activation up to C6 and generates inflammatory peptides, such as C5a and C5β, that could activate G proteins through specific receptors (36). Since activation of C7D produced minimal effect, G protein activation by serum complement was primarily due to the TCC assembly. As in DAG and ceramide generation (17), G protein activation was first noted with C5b-7 assembly, which increased further with C5b-8 and C5b-9 (Fig. 4). Although PTX-induced ADP-ribosylation of G proteins was much less in JY5 than JY25 (Figs. 5 and 7), increase in G protein activity by complement was similar between JY25 and JY5. This finding, together with the higher GTPyS binding activity in JY5, may be explained by the fact that TCC formation is four times more efficient in JY5 cells deficient in glycosylphosphatidylinositol-anchored proteins (17, 32).

Direct association of inserted TCC peptides with G proteins was demonstrated by the presence of PTX-sensitive Giα in immunoprecipitates obtained with anti-C5b-9 neoantigen, anti-C7, or anti-C8, but not with anti-C3 antibody from TCC-carrying cell or membrane lysates. When cells treated with heat-inactivated serum were immunoprecipitated with anti-C5b-9, PTX failed to induce Go ADP-ribosylation. The Go precipitated with TCC was a 41-kDa protein, migrating in an identical manner as the ADP-ribosylated Go by PTX in untreated plasma membranes. The capacity for PTX to ADP-ribosylate Go was reduced in membranes carrying preformed TCC. It is possible that certain amino acid residues of Go, that are targets for PTX after TCC interaction, may not be available for ADP-ribosylation in intact membranes due to steric hindrance, in contrast to easy accessibility of such amino acids in TCC-G protein complexes isolated from detergent lysates. This 41-kDa protein identified by ADP-ribosylation was reactive with antibodies specific for Giα1,2 and also with antibodies specific for Giα/Giα, indicating that TCC may interact with more than one G protein. The presence of Gα subunit in anti-C5b-9 immunoprecipitates suggested that G proteins complexed with TCC may exist as a trimeric form, that may also explain the ability of PTX to ADP-ribosylate Go after elution from the membrane. In addition, the isolated TCC-G protein complexes contained functionally active Ga, as shown by the capacity to bind GTPyS, which was blocked by PTX. The formation of molecular interaction between TCC peptides and G proteins is unknown.

Fig. 10 pretreatment of cells with PTX completely abolished the C5b-8 removal from the cell surface as demonstrated in this kinetic assay which determines the rate of C5b-8 elimination was not effective in blocking C5b-8 removal (Fig. 10).

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G Protein Activation by Terminal Complement Complexes

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