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CD19 IS A SUBSTRATE OF THE ANTIGEN RECEPTOR-ASSOCIATED PROTEIN TYROSINE KINASE IN HUMAN B CELLS

Chaim M. Roifman * **and Sui Ke**

Division of Immunology/Allergy, The Hospital for Sick Children,

Toronto, ON, M5G 1X8, Canada

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Summary: The B cell antigen receptor, mIg, is part of a multimolecular complex including Igα, Igβ, and CD19. We provide evidence here that upon ligation of mIg CD19 becomes phosphorylated on tyrosine residues. Further, protein tyrosine kinase lyn, which is activated by the antigen receptor, can be readily co-immunoprecipitated with CD19 suggesting this is the enzyme responsible for the antigen receptor mediated tyrosine phosphorylation of CD19.

Membrane Ig (mIg) mediates the clonally restricted recognition of antigen by the B cell and is, therefore, designated the B cell antigen receptor. mIg is part of a larger multimolecular complex (1-3) including the human Igα and Igβ; a 47 kDa and 37 kDa glycoproteins (4,5). Igα and Igβ molecules are the products of structurally related genes of the Ig superfamily, and they are believed to participate in the signal transduction process via the B cell antigen receptor (6). This assumption is mainly based on the finding that both Igα and Igβ are substrates of src-related protein tyrosine kinases which are associated with B cell antigen receptor (6). We, and others, have previously shown that B cell antigen receptor associated protein tyrosine kinase activity is necessary for activation of the phosphoinositide specific phospholipase C pathway and B cell proliferation (7-9). Recent studies have proposed that CD19, yet another member of the Ig superfamily, is associated with B cell antigen receptor and can mediate tyrosine phosphorylation (10). CD19 is also linked to the C3 complement receptor, CR2 (CD21), and three other membrane proteins (10).

We show here that CD19 is tyrosine phosphorylated after ligation of B cell antigen receptor with anti-IgM. CD19 may be a substrate of protein tyrosine kinase lyn which is

*To whom correspondence should be addressed.

Abbreviations: Igα - Immunoglobulin-α; Igβ - Immunoglobulin-β.

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physically associated with CD19 and undergoes autophosphorylation upon stimulation of B cell antigen receptor.

MATERIALS AND METHODS

Cell Preparation: Peripheral blood mononuclear cells (PBM) were from healthy volunteers. Mononuclear cells were isolated by Ficoll-Hypaque gradient. Adherent cells were removed by adherence to plastic dishes for 60 min at 37°C. Separation of T cells from B cells was accomplished by removing, by Ficoll-Hypaque centrifugation, the cells that rosetted with 2-aminoethyl-isothiouronium bromide-treated sheep erythrocytes as previously described. The resting B cell population expressed < 2% $CD3^+$ and > 98% $B1^+$ cells. All determinations of cell surface markers were determined by immunofluorescence staining on a flow cytometer (Epics V, Coulter Electronics, Hialeah, FL).

Reagents: ^{125}I-protein A was obtained from ICN. Monoclonal anti-phosphotyrosine antibodies affinity purified polyclonal and monoclonal antibodies against CD-19 (10) and antibodies against phosphotyrosine were purchased from Upstate Biotechnology Inc. NY.

Immunoprecipitation: 2 x 10^7 cells were incubated in RPMI-1640 for 5 to 30 min in the absence or presence of anti-IgM antibody. Cells were subsequently washed and resuspended in lysis buffer containing 20 mM Hepes (pH 7.4), 125 mM NaCl, 1% Triton X-100, 10 mM EDTA, 2 mM EGTA, 2 mM Na_3VO_4, 50 mM Sodium fluoride, 20 mM $ZnCl_2$, 10 mM Sodium pyrophosphate, 1 mM PMSF, 5 µg/ml leupeptin, 20 µg/ml Benzamidine, and centrifuged for 15 min at 12,000 xg. The supernatants were used as cell lysates. The lysates were incubated with the various antibodies at concentrations mentioned in the Legend To Figures per 0.5 ml of cell lysate for 1 h on ice and then with protein A-Sepharose CL4B for 1 h. Protein A-Sepharose CL4B was pretreated with rabbit anti-mouse immunoglobulin G (IgG). The Sepharose beads containing the immunoprecipitants were washed 3 times with washing buffer (as above except PMSF, leupeptin and Benzamidine were omitted), once with 0.5 M LiCl-0.1 M. This hydrochloride (pH 7.5) and once with 0.1 m NaCl - 1 mM EDTA - 2 mM Tris hydrochloride (pH 7.5). SDS-sample buffer was added to the washed immunoprecipitate and the samples were electrophoretically separated on an 8% SDS-polyacrylamide gel.

Electrophoresis and Western Blotting: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out as previously described and Western blotting analysis was performed essentially as described (7-9). B cells were stimulated as indicated, sedimented, and lysed with boiling sodium dodecyl sulfate (SDS) sample buffer. Proteins were separated on SDS-PAGE, transferred to nitrocellulose, and blotted with the anti-phosphotyrosine antibodies. Antibody reactivity was detected with ^{125}I-labeled protein A and autoradiography.

RESULTS AND DISCUSSION

We have studied the possibility that CD19 might be a substrate of the B cell antigen receptor mediated protein tyrosine kinase activity. Resting or anti-IgM stimulated human tonsillar B cells were lysed and immunoprecipitated with anti-CD19 antibody. Immune complexes were immunoblotted with anti-phosphotyrosine antibody. Fig 1 demonstrates that anti-IgM stimulated but not resting B cells shows a marked increase in tyrosine phosphorylation of a 95 kDa protein corresponding to the size of CD19. Tyrosine phosphorylation of CD19 was maximal as early as 1 min from stimulation of B cell antigen receptor and was sustained for at least 30 min.

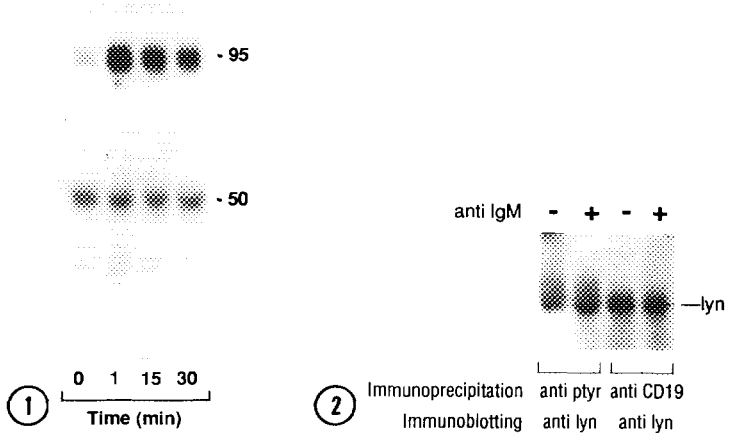

Figure 1. B cells (2 x 10^7) were incubated in the absence (Lane 1) or presence of anti-IgM antibody 20 μg/ml (Lanes 2-4) for various time periods as indicated. The lysates were immunoprecipitated with anti-CD19 antibody. The immunopellets were solubilized in SDS-sample buffer and electrophoretically separated on an 8% SDS-polyacrylamide gel. After Western blotting the phosphotyrosine containing proteins were detected using anti-phosphotyrosine antibody and ^{125}I-protein A. The results represent three separate experiments with similar results.

Figure 2. B cells (2 x 10^7) were incubated with or without anti-IgM antibody for 2 min. The lysates were immunoprecipitated with antibodies as indicated. Immunoblotting was performed with anti-lyn antibodies and ^{125}I-protein A. The results represent four separate experiments with similar results.

Next, we have attempted to determine which B cell antigen receptor linked protein tyrosine kinase of the src-family might be associated with CD19. Fig 2 shows lyn is tyrosine phosphorylated after mIg ligation and that CD19 immune complexes react with anti-lyn antibody. The results suggest that lyn kinase is the protein tyrosine kinase which is activated by B cell antigen receptor and is phosphorylating CD19 on tyrosine residues. Antibodies to other src-family protein tyrosine kinases or anti-syk antibody did not react with CD19 immune complexes (data not shown) suggesting they are not directly involved in CD19 phosphorylation.

We have shown here that CD19 is heavily phosphorylated on tyrosine residues after stimulation of B cell antigen receptor. The enzyme responsible for this phosphorylation is possibly the src-related lyn kinase because CD19 seems to be tidely associated with lyn even under rigid detergent conditions. However, our studies cannot exclude the possibility that yet another protein tyrosine kinase might be involved in phosphorylation of CD19.

CD19 which expressed on B lineage cells throughout their maturation from pre-B cell to a fully mature B cell seems to have a function in enhancing B cell antigen receptor induced B cell proliferation (11,12). Its extensive cytoplasmic tail suggest that this glycoprotein is involved in signal transduction. Indeed, ligation of CD19 with antibody raised against this molecule results in increased tyrosine phosphorylation and acute breakdown of phosphoinositides (10).

Our results indicate that like the B cell antigen receptor accessory molecules Igα and Igβ, CD19 is tyrosine phosphorylated suggesting that all these molecules participate as a receptor complex for signal transduction via the antigen receptor on B cells.

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