Claudia Schoch
Department of Internal Medicine III
University Hospital
Grosshadern
Ludwig-Maximilians-University
Munich, Germany

REFERENCES

1. Yin AH, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, Olweus J, Kearney J, Buck DW: AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood 90:5002, 1997

2. Miraglia S, Godfrey W, Yin AH, Atkins K, Warnke R, Holden JT, Bray RA, Waller EK, Buck DW: A novel five-transmembrane hematopoietic stem cell antigen: Isolation, characterization, and molecular cloning. Blood 90:5013, 1997

3. Mrozek K, Heinonen K, de la Chapelle A, Bloomfield CD: Clinical significance of cytogenetics in acute myeloid leukemia. Semin Oncol 24:17, 1997

4. Fruchart C, Lenormand B, Bastard C, Boulet D, Lesesve JF, Callat MP, Stamatoullas A, Monconduit M, Tilly H: Correlation between CD34 expression and chromosomal abnormalities but not clinical outcome in acute myeloid leukemia. Am J Hematol 5:175, 1996

Interleukin-8 in Acute Myeloid Leukemia

To the Editor:

In their recent report, Terui et al[1] describe the apoptotic activity exerted by endothelial interleukin-8 (IL-8) on a number of leukemic cell lines and in particular on K562 myeloid cells both in vitro and in vivo, in a mouse experimental model. The report by Terui et al[1] provides fascinating insights into the complex field of biological signals involved in cross-communication between leukemic and normal bystander cells. We would like to discuss a number of points emerging from their report, particularly its claim regarding "a new therapy for hematological malignancies."

Terui et al[1] were successful in inducing apoptosis in K562 as well as in other leukemic cell lines only in a minority of the overall cell population in each individual test in vitro (~20% of K562 cells underwent apoptosis). Moreover, they used a mouse model in which subcutaneous K562 tumors were partially suppressed by locally injected endothelial IL-8. Although these effects are biologically of great interest, they appear, in our opinion, either too limited or obtained in a setting too different from the usual pattern of leukemic growth, which only rarely presents as solid tumors, for us to be able to postulate substantial advantages in terms of leukemia treatment in vivo. Actually, the target for investigating the antileukemic effect of IL-8 should be primary leukemic cells. However, it may be of interest to remark that primary leukemia blasts can spontaneously produce IL-8[2,3] and express IL-8 receptors.[2] As far as acute myeloid leukemia (AML) is concerned, relevant amounts of IL-8 are produced by the great majority of AML with monocytic components (French-American-British [FAB] M4 and M5),[3,4] which usually respond poorly to therapy in terms of long-term leukemia control. Interestingly, monocytic blasts often localize in nonhematological tissues.[3,4] This phenomenon implies the ability to cross endothelial layers without undergoing apoptotic death, thus escaping the killing mechanisms described by Terui et al.[1] Theoretically, leukemic blasts may not only produce monocytic IL-8 in vivo, but even endothelial IL-8, as HL-60 cells do,[5] or even other N-terminus variants. Blast-derived IL-8 may be at least as important as exogenous IL-8 in terms of leukemia biology. If blast-derived IL-8 were simply a competitor for exogenous endothelial IL-8, this might suggest a limited role, if any, for a therapeutic approach based on the use of endothelial IL-8 in vivo. This point needs to be better clarified.

Fabrizio Vinante
Antonella Rigo
Marco A. Cassatella
Giovanni Pizzolo
Departments of Hematology and General Pathology
University of Verona
Verona, Italy

REFERENCES

1. Terui Y, Ikeda M, Tomizuka H, Kasahara T, Ohtsuki T, Uwai M, Mori M, Itoh T, Tanaka M, Yamada M, Shimamura S, Ishizaka Y, Ikeda K, Ozawa K, Miura Y, Hatake K: Activated endothelial cells induce apoptosis in leukemic cells by endothelial interleukin-8. Blood 92:2672, 1998

2. Tobler A, Moser B, Dewald B, Geiser T, Studer H, Baggiolini M, Fey MF: Constitutive expression of interleukin-8 and its receptor in human myeloid and lymphoid leukemia. Blood 82:2517, 1993

3. Vinante F, Rigo A, Vincenzi C, Ricetti MM, Marrocchella R, Chilosi M, Cassatella MA, Bonazzi L, Pizzolo G: IL-8 mRNA expression and IL-8 production by acute myeloid leukemia cells. Leukemia 7:1552, 1993

4. Vinante F, Rigo A, Tecchio C, Patuzzo C, Ricetti MM, Morosato L, Cassatella MA, Chilosi M, Pizzolo G: Preferential release of high amounts of interleukin-8 by myeloid blasts showing monocytic differentiation. Haematologica 81:195, 1996

5. Terui Y, Ikeda M, Tomizuka H, Kasahara T, Ohtsuki T, Uwai M, Mori M, Itoh T, Tanaka M, Yamada M, Shimamura S, Miura Y, Hatake K: Identification of a novel apoptosis-inducing factor derived from leukemic cells: Endothelial interleukin-8, but not monocyte-derived, induces apoptosis in leukemic cells. Biochem Biophys Res Commun 243:407, 1998

Association of CD10/Neutral Endopeptidase 24.11 With Membrane Microdomains Rich in Glycosylphosphatidylinositol-Anchored Proteins and Lyn Kinase

To the Editor:

Ganju et al[1] reported in *BLOOD* that the ectoenzyme CD10 (neutral endopeptidase 24.11, CALLA) expressed on the surface of lymphoid progenitors, mature granulocytes, and several nonhematopoietic cell types is associated with the protein tyrosine kinase (PTK) Lyn and with at least two other unidentified 40-kD and 75/80-kD phosphoproteins. These CD10-associated proteins (Lyn, p40, and

p75/80) become tyrosine phosphorylated under the conditions of in vitro kinase assay on CD10 immunoprecipitates obtained from detergent lysates of the pre-B–cell line Nalm-6. Ganju et al[1] suggest that coassociation between CD10 and a tyrosine phosphoprotein complex may link CD10 with so far poorly defined peptide-mediated signal transduction pathways and note that the mechanism of CD10 association with the Lyn-containing phosphoprotein complex is unclear because the CD10 cytoplasmic domain lacks characteristic structural motifs (SH2 and SH3 domains or their ligands) that could be responsible for such association. We offer here an explanation for these associations.

We observed very similar patterns of tyrosine phosphorylated protein zones (major 80 kD [so far unidentified], 55 kD [corresponding to autophosphorylated Lyn], and weak unidentified 40 kD) in vitro-kinase reactions of CD10 immunoprecipitates obtained from detergent lysates of pre-B–cell (Nalm-6) or B-cell (Ramos, Raji) lines. This pattern was identical to that obtained after in vitro kinase reactions of materials immunoprecipitated by monoclonal antibodies to some glycosphingolipids (eg, CDw75) and glycosylphosphatidylinositol (GPI)-anchored proteins (CD48, CD55; Fig 1A), indicating that all these molecules may be components of a common large complex. Indeed, preclearing of the Raji cell lysate by an anti-CD10 immunosorbent led to a substantial decrease of the kinase activity associated with CD48, CD55, and CDw75 immunoprecipitates (not shown). Moreover, a GPI-anchored protein, CD48, and the PTK Lyn could be readily demonstrated in the anti-CD10 immunoprecipitates (Fig 2). These multicomponent complexes were very large, as judged by gel chromatography on Sepharose 4B (Fig 1B), and buoyant, as judged by sucrose gradient ultracentrifugation (Fig 1C). The presence of a fraction of CD10 in these large complexes (together with Lyn and GPI-anchored molecules) could be demonstrated by Western blotting (Fig 3). Therefore, we conclude that a fraction of CD10 is a component of large, detergent-resistant "GPI-microdomains"[2] (also called glycolipid rafts,[3] glycolipid-enriched membranes [GEM],[4] or detergent-insoluble glycosphingolipid complexes [DIG][3]), membrane specializations rich in cholesterol, glycosphingolipids, GPI-anchored proteins, PTKs of the Src family, and some other signaling molecules but devoid of most transmembrane proteins.[2-4] These membrane microdomains appear to be involved in signaling via GPI-anchored proteins[5] and glycolipids,[6] but may play an essential role also in signaling via Fc-receptors[7] and TCR.[8] CD10 appears to be one of very few transmembrane proteins present in these microdomains. It is not clear what determines whether a transmembrane protein can be a component of the GPI-microdomains, but posttranslational modification by palmitylation is a likely possibility; actually, a slight difference in electrophoretic mobility of CD10 present in the buoyant microdomains versus that excluded from them (Fig 3) is compatible with such a modification.

It can be speculated that the fraction of CD10 incorporated in the GPI-microdomains and thus potentially capable of making use of the

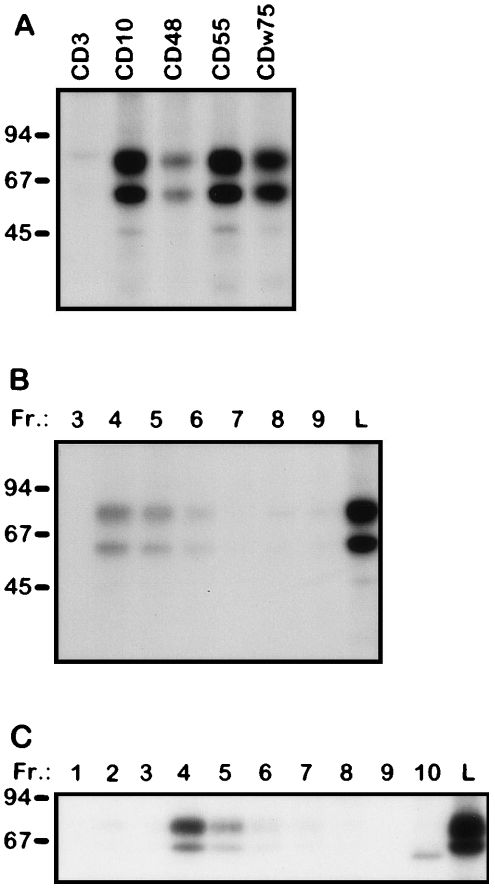

Fig 1. (A) The results of in vitro kinase assays on the indicated immunoprecipitates obtained from NP40-solubilized Raji cells (analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE] and autoradiography). Note major in vitro phosphorylated zones of 75-80 kD and 55-60 kD similar to those observed by Ganju et al.[1] The monoclonal antibodies used were MEM-57 (CD3; negative control), MEM-78 (CD10), MEM-102 (CD48), MEM-118 (CD55), and HH2 (CDw75). (B) The results of in vitro kinase assays on CD10 immunoprecipitates obtained from the detergent lysate of Raji cells size fractionated on Sepharose 4B column (fractions 3-9; L is the unfractionated lysate). Fractions 3 and 4 represent void volume where very large complexes or particles elute; elution volume of size standards IgM and IgG was in fractions 6 and 8, respectively. (C) Same as in (B) but the lysate was fractionated by sucrose density gradient ultracentrifugation (gradient from 40% to 5% sucrose). Fraction 4 (maximum of CD10-associated kinase activity) corresponds to buoyant density of 1.05 to 1.09 g/mL. An identical profile of separation was observed in the case of CD48 and CD55 immunoprecipitates (not shown).

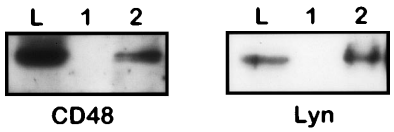

Fig 2. Coisolation of CD48 and Lyn with CD10 from 1% NP40 lysate of Nalm-6 cells. The immunoprecipitates obtained from an irrelevant control (1) or anti-CD10 (2) immunosorbents and the original lysate were subjected to SDS-PAGE/Western blotting and stained by antibodies to CD48 and Lyn, respectively.

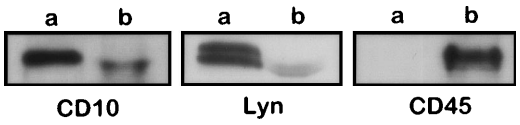

Fig 3. Western blotting analysis of the fractions obtained by sucrose gradient ultracentrifugation of Raji cell detergent lysate. The blots were immunostained by Abs to CD10, Lyn, and CD45 (a control protein that is not a component of the large buoyant complexes). (a) The buoyant fraction from the top of the gradient; (b) the bottom fraction. The buoyant fraction contained also large amounts of GPI-anchored proteins (CD48, CD55, and CD59; not shown).

associated signaling molecules may have unique functions in addition to the well-known ectoenzyme activity.

ACKNOWLEDGMENT

This work was supported by Grant No. 310/96/0673 from the Grant Agency of the Czech Republic. V.H. is supported by an International Research Scholar's award from the Howard Hughes Medical Institute.

Pavla Angelisová
Karel Drbal
Václav Hořejší
Institute of Molecular Genetics
Academy of Sciences of the Czech Republic
Prague, Czech Republic
Jan Černý
Faculty of Sciences
Charles University
Prague, Czech Republic

REFERENCES

1. Ganju RK, Shpektor RG, Brenner DG, Shipp MA: CD10/neutral endopeptidase 24.11 is phosphorylated by casein kinase II and coassociates with other phosphoproteins including the Lyn Src-related kinase. Blood 88:4159, 1996

2. Cinek T, Hořejší V: The nature of large noncovalent complexes containing glycosyl-phosphatidylinositol-anchored membrane glycoproteins and protein tyrosine kinases. J Immunol 149:2262, 1992

3. Simons K, Ikonen E: Functional rafts in cell membranes. Nature 387:569, 1997

4. Rodgers W, Crise B, Rose JK: Signals determining protein tyrosine kinase and glycosyl-phosphatidylinositol-anchored targeting to a glycolipid-enriched membrane fraction. Mol Cell Biol 14:5384, 1994

5. Štefanová, I, Hořejší V, Ansotegui IJ, Knapp W, Stockinger H: GPI-anchored cell-surface molecules complexed to protein tyrosine kinases. Science 254:1016, 1991

6. Iwabuchi K, Yamamura S, Prinetti A, Handa K, Hakomori S: GM3-enriched microdomain involved in cell adhesion and signal transduction through carbohydrate-carbohydrate interaction in mouse melanoma B16 cells. J Biol Chem 273:9130, 1998

7. Field KA, Holowka D, Baird B: Compartmentalized activation of the high affinity immunoglobulin E receptor within membrane domains. J Biol Chem 272:4276, 1997

8. Xavier R, Brennan T, Li Q, McCormack C, Seed B: Membrane compartmentation is required for efficient T cell activation. Immunity 8:723, 1998