Correspondence

To the editor:

Is active acid sphingomyelinase required for the antiproliferative response to rituximab?

Combination of conventional chemotherapy and the anti-CD20 chimeric monoclonal antibody rituximab has become the standard of care for treating B-cell malignancies.[1,2] Whereas the precise mechanisms of action of rituximab in vivo remain ill-defined, its effectiveness likely involves complement- and antibody-dependent cytotoxicity.[3] Bezombes et al reported that acid sphingomyelinase (aSMase) and its product ceramide may mediate the growth inhibitory effect of rituximab on malignant B cells.[4] aSMase is a hydrolase, encoded by the *SMPD1* gene, which cleaves the ubiquitous phospholipid sphingomyelin to release ceramide.[5] This lipid has emerged as a bioactive molecule that modulates membrane properties and the response of cancer cells to a variety of insults, including classic chemotherapeutic agents.[5,6] Bezombes et al suggested that the generation of ceramide in lymphoma and B-CLL cells treated with rituximab occurred through aSMase; both ceramide and aSMase colocalized with CD20 at the cell surface. These events were proposed to mediate the in vitro growth inhibition.[4] Intriguingly, rituximab was reported to interact with a peptide derived from a protein sharing homology with aSMase, suggesting that rituximab may bind to this enzyme.[7] Whether such a function of aSMase in the response to rituximab translates to the in vivo situation in patients with lymphoma was unknown.

We had the unique opportunity to investigate the role of aSMase in an adult male patient with genetic aSMase deficiency who developed a marginal zone lymphoma at age 65. After 5 cycles of a combination of rituximab, cyclophosphamide, vincristine, and prednisone, splenomegaly was still present, leading to treatment interruption and splenectomy. Unexpectedly, microscopic examina-

tion of the spleen revealed no lymphoma cells but foamy cells suggestive of an inherited lipid storage disorder. Deficient aSMase activity was demonstrated by enzyme studies on peripheral leukocytes (0.14 vs 0.61 nmol/h/mg protein in controls), spleen (0.03 vs 0.68 nmol/h/mg) and EBV-transformed lymphoid cells (0.22 vs 2.6 nmol/h/mg). Diagnosis of Niemann-Pick disease (NPD; type B) was further confirmed by documenting sphingomyelin storage in spleen (389 vs 52 nmol/mg in controls) and identification of 2 point mutations in the *SMPD1* gene (c.551C>T, p.P184L and c.733G>A, p.G245S), which have already been described in patients with NPD type B.[8,9] Thus, despite aSMase deficiency, the patient efficiently responded to rituximab combined with chemotherapy. No relapse was observed 24 months after treatment initiation.

We then wanted to assess the in vitro response of NPD B cells to rituximab. As fresh lymphoma cells from the patient were not available, EBV-transformed lymphoid cells from the patient, as well as from other patients with NPD type A or B, and from control individuals, were tested. As illustrated in Figure 1, aSMase-deficient cells were equally sensitive to the antiproliferative effects of rituximab (and the chemotherapeutic agents used; not shown).

Therefore, this case report suggests that aSMase is dispensable for rituximab efficacy. If not fortuitous, the association of lymphoma and NPD would be reminiscent of the increased risk of malignancies seen in patients with Gaucher disease,[10] another lipid storage disorder, thus pointing to the possible role of aSMase deficiency in cancer development.
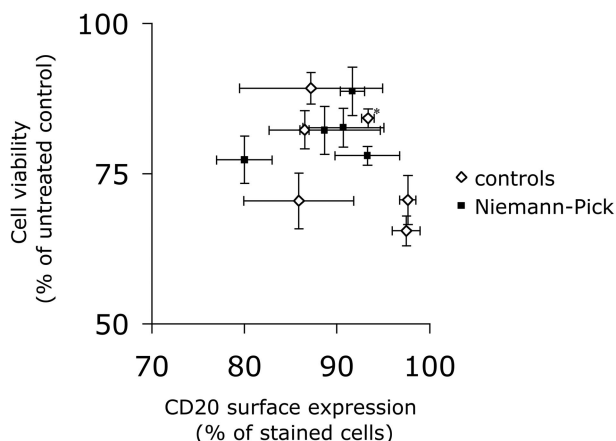
Figure 1. Sensitivity of control and Niemann-Pick disease cells to rituximab. EBV-transformed lymphoid cells from NPD patients (including the patient herein described, a patient with NPD type A and 3 patients with NPD type B) and from 6 control subjects (including a cell line from one of the NPD type B patients that had been corrected by retroviral transfer of the *SMPD1* cDNA; denoted with an asterisk) were incubated for 72 hours with rituximab (10 μg/mL). Cell viability was assessed by the MTT test and is expressed as a function of cell-surface expression of CD20, as determined by flow cytometry. Data are means ± SEM of at least 3 independent determinations. Similar results were obtained in the presence of non-decomplemented serum (not shown). In addition, analysis of cell cycle by flow cytometry revealed a slight increase in the sub-G_1 population both in control and NPD cells treated with rituximab (not shown).

Frédérique Sabourdy
*Service de Biochimie-Institut Fédératif de Biologie, CHU Purpan,
and INSERM UMR 1037, Centre de Recherche en Cancérologie de Toulouse,
and Université Paul Sabatier,
Toulouse, France*

Janick Selves
*Service d'Anatomo-Pathologie Purpan, CHU Purpan,
and INSERM UMR 1037, Centre de Recherche en Cancérologie de Toulouse,
and Université Paul Sabatier,
Toulouse, France*

Leonardo Astudillo
*Service de Médecine Interne, CHU Purpan,
and INSERM UMR 1037, Centre de Recherche en Cancérologie de Toulouse,
and Université Paul Sabatier,
Toulouse, France*

Camille Laurent
*Service d'Anatomo-Pathologie Purpan, CHU Purpan,
and INSERM UMR 1037, Centre de Recherche en Cancérologie de Toulouse,
and Université Paul Sabatier,
Toulouse, France*

Pierre Brousset
*Service d'Anatomo-Pathologie, CHU Purpan,
and INSERM UMR 1037, Centre de Recherche en Cancérologie de Toulouse,
and Université Paul Sabatier,
Toulouse, France*

Marie-Bernadette Delisle
*Service d'Anatomo-Pathologie, CHU Rangueil,
and INSERM UMR 1037, Centre de Recherche en Cancérologie de Toulouse,
and Université Paul Sabatier,
Toulouse, France*

Nicole Therville
*INSERM UMR 1037, Centre de Recherche en Cancérologie de Toulouse,
Toulouse, France*

Nathalie Andrieu-Abadie
*INSERM UMR 1037, Centre de Recherche en Cancérologie de Toulouse,
Toulouse, France*

Bruno Ségui
*INSERM UMR 1037, Centre de Recherche en Cancérologie de Toulouse,
and Université Paul Sabatier,
Toulouse, France*

Christian Recher
*Service d'Hématologie, CHU Purpan,
and INSERM UMR 1037, Centre de Recherche en Cancérologie de Toulouse,
and Université Paul Sabatier,
Toulouse, France*

Thierry Levade
*Service de Biochimie-Institut Fédératif de Biologie, CHU Purpan,
and INSERM UMR 1037, Centre de Recherche en Cancérologie de Toulouse,
and Université Paul Sabatier,
Toulouse, France*

References

1. Molina A. A decade of rituximab: improving survival outcomes in non-Hodgkin's lymphoma. *Annu Rev Med.* 2008;59:237-250.

2. Michallet AS, Coiffier B. Recent developments in the treatment of aggressive non-Hodgkin lymphoma. *Blood Rev.* 2009;23(1):11-23.

3. Beers SA, Chan CH, French RR, et al. CD20 as a target for therapeutic type I and II monoclonal antibodies. *Semin Hematol.* 2010;47(2):107-114.

4. Bezombes C, Grazide S, Garret C, et al. Rituximab antiproliferative effect in B-lymphoma cells is associated with acid-sphingomyelinase activation in raft microdomains. *Blood.* 2004;104(4):1166-1173.

5. Smith EL, Schuchman EH. The unexpected role of acid sphingomyelinase in cell death and the pathophysiology of common diseases. *FASEB J.* 2008; 22(10):3419-3431.

6. Ogretmen B, Hannun YA. Biologically active sphingolipids in cancer pathogenesis and treatment. *Nat Rev Cancer.* 2004;4(8):604-616.

7. Perosa F, Favoino E, Caragnano MA, Dammacco F. Generation of biologically active linear and cyclic peptides has revealed a unique fine specificity of rituximab and its possible cross-reactivity with acid sphingomyelinase-like phosphodiesterase 3b precursor. *Blood.* 2006;107(3):1070-1077.

8. Pavlu-Pereira H, Asfaw B, Poupctová H, et al. Acid sphingomyelinase deficiency. Phenotype variability with prevalence of intermediate phenotype in a series of twenty-five Czech and Slovak patients. A multi-approach study. *J Inherit Metab Dis.* 2005;28(2):203-227.

9. Simonaro CM, Desnick RJ, McGovern MM, Wasserstein MP, Schuchman EH. The demographics and distribution of type B Niemann-Pick disease: novel mutations lead to new genotype/phenotype correlations. *Am J Hum Genet.* 2002; 71(6):1413-1419.

10. de Fost M, Vom Dahl S, Weverling GJ, et al. Increased incidence of cancer in adult Gaucher disease in Western Europe. *Blood Cells Mol Dis.* 2006;36(1):53-58.

To the editor:

Differential prognosis impact of *IDH2* mutations in cytogenetically normal acute myeloid leukemia

Mutations in the genes isocitrate dehydrogenase 1 and 2 (*IDH1/IDH2*) have been recently identified in gliomas and acute myeloid leukemia (AML).[1] We and others reported that *IDH1*[R132] mutations were associated with normal karyotype and nucleophosmin 1 gene (*NPM1*) mutations. In a large cohort of 1450 AML patients, Schnittger et al showed a general unfavorable impact of *IDH1* mutation in AML.[2] Other studies, focused on cytogenetically normal (CN) AML, suggested that this effect would be principally observed in CN-AML with favorable genotype, that is, with *NPM1* mutation without internal tandem duplication of *FLT3* gene (*FLT3-ITD*).[3-6]

The prognosis impact of *IDH2* mutations is not clearly defined. Two major host spots of *IDH2* mutations have been reported in AML. *IDH2*[R172] mutations, homologous to *IDH1*[R132] mutations,

Table 1. Frequency and prognostic impact of *IDH2* gene mutations in CN-AML

Reference	n	Age range, y	*IDH2*[R172] mutation		*IDH2*[R140] mutation	
			Frequency	Prognosis	Frequency	Prognosis
Marcucci et al[3]	358	19-83	4%	↓ CR	16%	NC
Thol et al[7]	272	≤ 60	1.1%	No impact†	11%	No impact†
Paschka et al[4]	338	16-60	NC	NC	NC	NC
Boissel et al[5]	213	17-70	6%	↓ CR, ↑ RR, ↓ OS	—	—
Abbas et al[8]	353	15-77	14%†	No impact†	14%†	No impact†
Chou et al[9]	155‡	18-90	NC	↑ OS	NC	↑ OS

ND indicates not done; NC, not communicated; CR, complete remission; OS, overall survival; —, not done; and RR, risk of relapse.

*All *IDH* mutations.

†All *IDH2* mutations.

‡Patients with prognosis analysis.