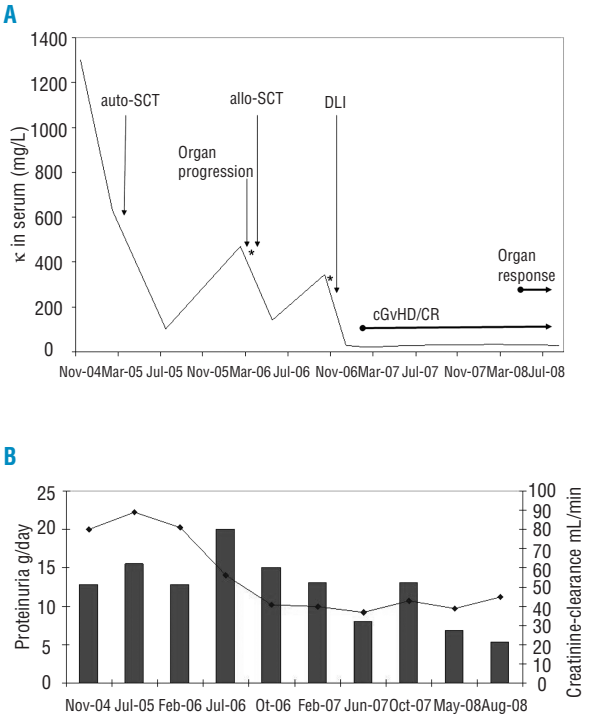

Figure 1. Description of hematologic and organ response in Patient 1 (AL κ+). (A) FLC κ during treatment course. *2 cycles of bortezomib were administered for re-induction before allo-SCT and DLI. (B) Illustration of kidney response. Bars denote proteinuria and the line the creatinine-clearance. A 50% reduction of proteinuria was achieved since May 2008. cGvHD: chronic graft-versus-host-disease; CR: complete remission with conversion to 100% donor plasma cell chimerism; DLI: donor lymphocyte infusion; FLC: free-light chain.

clonal plasma cell burden is not as high and the plasma cells are considered to be more indolent in AL amyloidosis.[12] However, as shown in MM this potent graft-versus-plasma-cell-dyscrasia-effect is also associated with the occurrence of chronic GvHD.

In summary, our report provides the rationale to further investigate allo-SCT and post-transplant immunotherapeutic strategies in systemic AL amyloidosis.

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Key words: AL amyloidosis, multiple myeloma and other Plasma Cell Dyscrasias; allogeneic transplantation, donor lymphocyte infusion, plasma cell chimerism.

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References

1. Skinner M, Sanchorawala V, Seldin DC, Dember LM, Falk RH, Berk JL, et al. High-dose melphalan and autologous stem-cell transplantation in patients with AL amyloidosis: an 8-year study. Ann Intern Med 2004;140:85-93.
2. Palladini G, Perfetti V, Obici L, Caccialanza R, Semino A, Adami F, et al. Association of melphalan and high-dose dexamethasone is effective and well tolerated in patients with AL (primary) amyloidosis who are ineligible for stem cell transplantation. Blood 2004;103:2936-8.
3. Sanchorawala V, Wright DG, Rosenzweig M, Finn KT, Fennessey S, Zeldis JB, et al. Lenalidomide and dexamethasone in the treatment of AL amyloidosis: results of a phase 2 trial. Blood 2007;109:492-6.
4. Dispenzieri A, Lacy MQ, Zeldenrust SR, Hayman SR, Kumar SK, Geyer SM, et al. The activity of lenalidomide with or without dexamethasone in patients with primary systemic amyloidosis. Blood 2007;109:465-70.
5. Kastritis E, Anagnostopoulos A, Roussou M, Toumanidis S, Pamboukas C, Migkou M, et al. Treatment of light chain (AL) amyloidosis with the combination of bortezomib and dexamethasone. Haematologica 2007;92:1351-8.
6. Sanchorawala V, Skinner M, Quillen K, Finn KT, Doros G, Seldin DC. Long-term outcome of patients with AL amyloidosis treated with high-dose melphalan and stem-cell transplantation. Blood 2007;110:3561-3.
7. Schonland SO, Lokhorst H, Buzyn A, Leblond V, Hegenbart U, Bandini G, et al. Allogeneic and syngeneic hematopoietic cell transplantation in patients with amyloid light-chain amyloidosis: a report from the European Group for Blood and Marrow Transplantation. Blood 2006;107:2578-84.
8. Kroger N, Kruger W, Renges H, Zabelina T, Stute N, Jung R, et al. Donor lymphocyte infusion enhances remission status in patients with persistent disease after allografting for multiple myeloma. Br J Haematol 2001;112:421-3.
9. Gertz MA, Comenzo R, Falk RH, Fermand JP, Hazenberg BP, Hawkins PN, et al. Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis, Tours, France, 18-22 April 2004. Am J Hematol 2005;79:319-28.
10. Kroger N, Zagrivnaja M, Schwartz S, Badbaran A, Zabelina T, Lioznov M, et al. Kinetics of plasma-cell chimerism after allogeneic stem cell transplantation by highly sensitive real-time PCR based on sequence polymorphism and its value to quantify minimal residual disease in patients with multiple myeloma. Exp Hematol 2006;34:688-94.
11. Crawley C, Iacobelli S, Bjorkstrand B, Apperley JF, Niederwieser D, Gahrton G. Reduced-intensity conditioning for myeloma: lower nonrelapse mortality but higher relapse rates compared with myeloablative conditioning. Blood 2007;109:3588-94.
12. Bochtler T, Hegenbart U, Cremer FW, Heiss C, Benner A, Hose D, et al. Evaluation of the cytogenetic aberration pattern in amyloid light chain amyloidosis as compared to monoclonal gammopathy of undetermined significance reveals common pathways of karyotypic instability. Blood 2008;111:4700-5.

In HPA 1a-immunized women the decrease in anti-HPA 1a antibody level during pregnancy is not associated with anti-idiotypic antibodies

A large screening and intervention study, aimed at reducing morbidity and mortality associated with severe anti-HPA 1a antibody induced neonatal alloimmune thrombocytopenia (NAIT), has recently been carried out in Norway.[1] Recently, it was shown that the anti-HPA 1a levels surprisingly decreased in 92 of 147 women who had been pregnant previously and who carried an HPA 1a positive fetus ($P_{92 \text{ or more of } 147}=0.003$).[2]

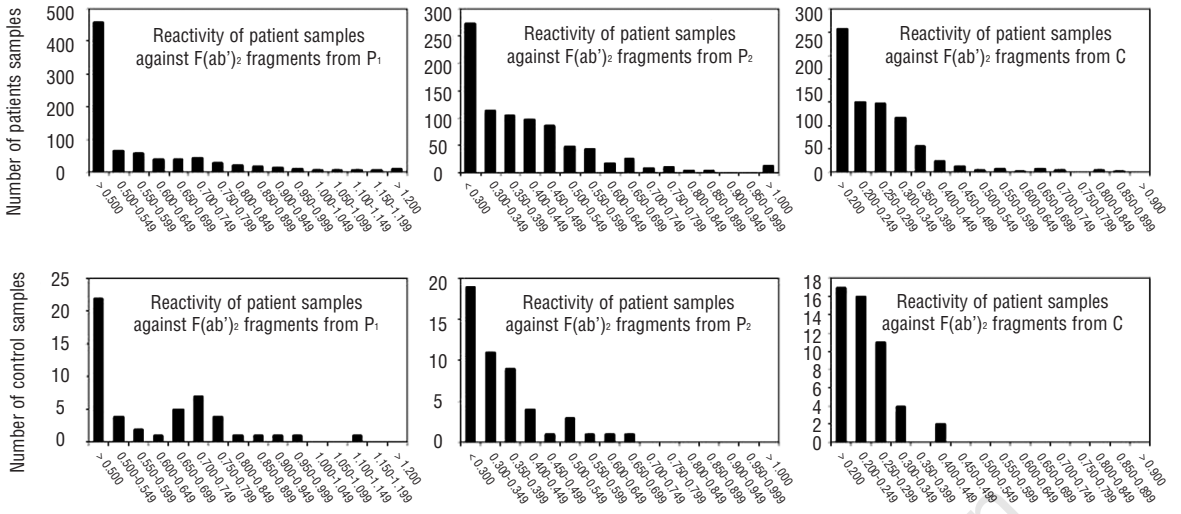

Figure 1. The frequency distribution of patient and control samples. The anti-idiotypic reactivities (OD at 405 nm) against F(ab')2 fragments from the two HPA 1a-immunized women (P1 and P2) and the healthy control (C) are shown.

The reason for decline in anti-HPA 1a antibodies during pregnancy in multigravida is not known. One mechanism by which the antibody repertoire can be regulated is via idiotypic networks. According to this concept an HPA 1a-immunized woman may develop anti-idiotypic antibodies (usually designated Ab_2) which are antibodies against antigenic determinants (idiotopes) on the variable region of the anti-HPA 1a antibodies. These anti-idiotypic antibodies may play an important immuno-regulatory role as they can blunt the initial immune response (Ab_1).[3,4] We therefore examined whether the observed decline in anti-HPA 1a antibody level in immunized pregnant women was associated with a concurrent increase in anti-idiotypic antibodies. The study was approved by the Regional Committee for Medical Research Ethics, North Norway (approval n. P-REK V 13/1995).

A total number of 829 samples of EDTA plasma were collected from 157 HPA 1a-incompatible pregnancies included in the screening and intervention study.[1] As controls we used 18 samples collected during pregnancy in 4 non-HPA 1a-immunized pregnant women, and 28 samples from normal blood donors (11 males and 17 females). The labeling system for the control samples was similar to the system used for the patients' samples. The coding (patients versus controls) was concealed until all analyses were completed.

Anti-idiotypic activity was assessed as previously described.[5] Briefly, IgG was purified from 2 HPA 1a immunized women (P_1 and P_2) and from one non-immunized healthy control (C). $F(ab')_2$ fragments (from P_1, P_2 and C) prepared by pepsin digestion were used as coating proteins in an enzyme-linked immunosorbent assay (ELISA) for detection of anti-idiotypic antibodies (Ab_2) in plasma from patients and controls.

On each ELISA plate four different dilutions of immunoglobulin (100, 50, 25 and 12.5 μg/mL; Gamunex, Talecris Biotherapeutics, Mississauga, ON, Canada) as well as plasma samples from 2 of 4 healthy individuals were included as controls. The results from these healthy individuals were not analyzed in a blinded fashion, and hence they were not included in the statistical analysis. All samples from individual pregnant women were analyzed on one ELISA plate.

There was no significant difference in anti-idiotypic reactivity between samples from HPA 1a-immunized women and controls. There was no significant difference in the dispersion of anti-idiotypic reactivity between the study objects and the controls and no obvious difference in the frequency distribution pattern of anti-idiotypic reactivity between study objects and controls (Figure 1), indicating that the observed reactivity was not directed against the anti-HPA 1a specific $F(ab')_2$ fragments. When the analysis was restricted to those women in whom there was a decrease in anti-HPA 1a level during pregnancy, we again could not find a concurrent increase in anti-idiotypic reactivity.

Our results contrast with a previous report suggesting that anti-idiotypic networks play a pivotal role in regulation of anti-HLA antibody levels. Atlas *et al*. showed that 55 of 82 multitransfused HLA immunized patients with decreasing anti-HLA antibody levels over time, had concurrently increasing levels of anti-idiotypic antibodies in their sera.[6] Anti-idiotypic antibodies could not be found in sera from patients with persistently high anti-HLA antibody levels.[6] In addition, more than one third of the anti-idiotypic antibodies inhibited the binding of the anti-HLA antibodies to platelets, indicating that they were specific for the paratopes of the anti-HLA andibodies.[6]

One possible explanation for the discrepancy between our results and those reported by Atlas *et al*. is that alloimmunization in NAIT is caused by a point mutation where a single nucleotide substitution results in one amino acid replacement at position 33 in GPIIIa (from proline in HPA 1b to leucine in HPA 1a), whereas in HLA-alloimmunization the antigenic diversity between different HLA molecules is much larger. Consequently the antibody repertoire of anti-HLA antibodies is considerably larger than that of anti-HPA 1a antibodies and perhaps the latter antibodies (Ab1) cannot effectively generate the production of anti-idiotypic antibodies (Ab2).

In conclusion, it is unlikely that idiotypic regulation of anti-HPA 1a antibodies occurs during pregnancy in HPA 1a-immunized women.

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Key words: fetal and neonatal alloimmune thrombocytopenia, HPA 1a, anti-idiotypic antibodies, pregnancy.

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References

1. Kjeldsen-Kragh J, Killie MK, Tomter G, Golebiowska E, Randen I, Hauge R, et al. A screening and intervention program aimed to reduce mortality and serious morbidity associated with severe neonatal alloimmune thrombocytopenia. Blood 2007;110:833-9.
2. Killie MK, Husebekk A, Kjeldsen-Kragh J, Skogen B. A prospective study of maternal anti-HPA 1a antibody level as a potential predictor of alloimmune thrombocytopenia in the newborn. Haematologica 2008;93:870-7.
3. Rodey GE. Anti-idiotypic antibodies and regulation of immune responses. Transfusion 1992;32:361-76.
4. Shoenfeld Y. The idiotypic network in autoimmunity: antibodies that bind antibodies that bind antibodies. Nat Med 2004;10:17-8.
5. Semple JW, Kim M, Lazarus AH, Freedman J. Gammaglobulins prepared from sera of multiparous women bind anti-HLA antibodies and inhibit an established in vivo human alloimmune response. Blood 2002;100:1055-9.
6. Atlas E, Freedman J, Blanchette V, Kazatchkine MD, Semple JW. Downregulation of the anti-HLA alloimmune response by variable region-reactive (anti-idiotypic) antibodies in leukemic patients transfused with platelet concentrates. Blood 1993;81:538-42.

Slow responses to standard dose rituximab in immune thrombocytopenic purpura

We read with interest the article by Zaja *et al*. in which the results of a prospective multicenter Phase II study to assess the response rates of lower dose rituximab in adults with chronic immune thrombocytopenic purpura (ITP) were reported.[1] In this single arm study 28 ITP patients received rituximab (100 mg/m^2) weekly for four weeks. An overall response (platelet count >50×10^9/L) and complete response (platelet count >100×10^9/L) was achieved in 21/28 (75%) and 12/28 (43%) of patients, respectively. Interestingly the time to treatment response with lower dose rituximab was longer than in published studies with standard dose (375mg/m^2 weekly for four weeks).[2-4] The median time to a complete response was 44 days with a range of 7-90 days.

In a recent prospective Phase II study which assessed the efficacy of standard dose rituximab in chronic adult ITP patients, most patients who responded to rituximab did so early and none of the patients who failed to reach a platelet count of 50×10^9//L in two weeks achieved a good response at one year.[2]

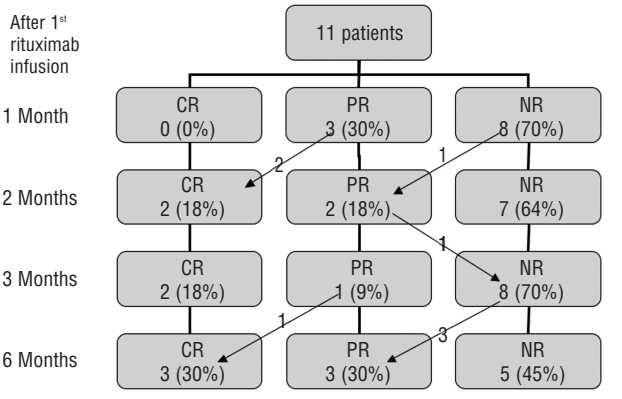

Figure 1. Response of chronic ITP patients over time, following first rituximab infusion. Complete response (CR), platelet count >100×10^9/L partial response (PR), platelet count 50-100×10^9/L. No response (NR). The arrows and overlying numbers represent patients changing response. Patients who required alternative treatment were classified as having no response.

In our center we have treated 11 patients (6 female and 5 male, mean age 50) with refractory chronic ITP with standard dose rituximab (375 mg/m^2 weekly for four weeks) with similar response rates (6 patients reached a platelet count of >50×10^9/L, including 3 >100×10^9/L at six months) but we have noted that delayed responses to standard dose rituximab also occur (Figure 1). The best responses were seen in 2 female patients, aged 32 and 26 years, with baseline platelet counts of 4 and 25×10^9/L. Their platelet counts at one month were 87 and 84×10^9/L and at six months were 521 and 230×10^9/L, respectively without any further treatment. In fact in our experience, at one month, no patient had platelets > 100×10^9/L (Figure 1). In a systemic review, complete responses usually occurred 3-8 weeks after the first infusion of rituximab.[5] However, our data show that delayed responses to standard dose rituximab can occur. These responses are unlikely to reflect spontaneous remission as this rarely occurs in adult chronic ITP.[6]

Splenectomy has been considered standard second-line therapy for ITP.[7] The response rate is about 65% but it is associated with a mortality of 0.2-1% and morbidity of 9.6-12.9% depending on the age of the patient and technique used.[8] There is accumulating evidence that rituximab can be a safe and effective way to defer splenectomy particularly in younger patients.[9] A lower dose rituximab regimen would result in considerable cost savings compared to a standard dose regimen and may be associated with less transfusion related reactions. While delayed responses may occur in the lower dose regimen they may also occur with standard dose rituximab. Since maximal response to both dosing regimens may be delayed, a decision regarding splenectomy should not be made until at least six months after rituximab therapy.

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Key Words: platelets, immune thrombocytopenic purpura, disorders of platelet function.