

To the editor:

Variable patterns of response to rituximab treatment in adults with chronic idiopathic thrombocytopenic purpura

In a recent issue of *Blood* we reported that rituximab anti-CD20 monoclonal antibody is an active drug in adults with chronic idiopathic thrombocytopenic purpura (ITP), that it is able to induce long-lasting responses, and that in all responders a rise of the platelet count occurs early during treatment, usually right after the first antibody administration.¹ We have now expanded our series with 7 additional cases, in whom we observed somewhat different patterns of response. Because patients with chronic ITP are being recruited for phase 2 clinical trials with rituximab (browse the web sites <http://www.clinicaltrials.gov> and <http://www.itppeople.com/clinical.htm> for more information), we would like to describe these cases.

Patients' pretreatment characteristics are reported in Table 1. There were 6 women and 1 man, with a median age of 40 years (range, 20-66 years). All cases had ITP that had been resistant to between 2 and 6 different therapeutic regimens, including 3 patients who had already failed splenectomy. Rituximab schedule (375 mg/m² intravenously once weekly for 4 consecutive weeks) and response criteria have been described before.¹

All patients concluded the 4 doses of treatment and could be evaluated for response. As illustrated in Table 1, the platelet count rose to greater than 50 × 10⁹/L in 6 patients, with 4 achieving a complete response (platelets > 100 × 10⁹/L) and 2 a partial response (platelets > 50 × 10⁹/L). In 5 of the responders (cases 1, 2, 4, 5, and 7) there was a marginal or no increase of the platelet count during rituximab administration, with responses appearing only 2 to 5 weeks after the last antibody infusion. Peak platelet counts occurred 10 to 16 weeks after the start of treatment, with a median peak count of 408 × 10⁹/L (range, 92 × 10⁹/L to 751 × 10⁹/L). Two patients with complete remission (CR) have remained in remission with stable platelet counts during follow-up intervals of 7 to 12 or more months after the end of treatment. In the other 3 responders the follow-up is less than 6 months.

In one patient (case 6), we observed an early but transient rise of the platelet count after the first dose of rituximab, with a late response beginning to appear at week 8 from the start of treatment and lasting 7 weeks.

There were no significant changes of serum IgG, IgA, and IgM levels throughout the study, whereas peripheral blood B-cell counts sharply declined to near-zero values after the first dose of rituximab. Median absolute T-cell counts as well as natural-killer cell counts remained stable during the study period. No patient experienced adverse events during therapy.

Combining the results of our initial report with the present data, we can identify 2 patterns of response, early and late. In early responders there is an increase of the platelet count after the first or second antibody infusion. The platelet count continues to rise thereafter until a peak count, which is usually observed between week 6 and week 10. In late responders there is no rise of the platelet count during rituximab administration, or there is just a marginal and transient increase; in these cases platelets begin to rise at weeks 6 to 8 and very rapidly reach a peak count. How can one explain these 2 patterns of response? With the available data one can only speculate. It is possible that

Table 1. Clinical and hematologic characteristics of patients with chronic ITP

Case no.	Age	Sex	Prior therapy	Duration of disease (mo)	Platelet count prior to rituximab (× 10 ⁹ /L)*	Platelet counts after rituximab (× 10 ⁹ /L)																Response duration (weeks)	Additional therapy during the study period	Additional therapy during the observation phase after rituximab treatment	Subsequent therapy
						Week 1	Week 2	Week 3	Week 4	Week 6	Week 8	Week 10	Week 12	Week 16	Week 18	Week 20	Week 24	Week 28	Week 32	Week 36	Week 40				
1	20	F	P, Ig, DXM, aRh, S	12	2	2	2	6	4	3	4	4	4	4	4	121	408	385	392	56+	P†				
2	50	F	P, Ig, DXM, aRh, S, V	84	1	2	2	8	12	6	6	6	6	6	77	120	131	123	28+	P†					
3	40	F	P, Ig	36	10	38	21	5	9	9	7	7	7	7	25	6	6	6	—	P†	P	P‡			
4	32	F	P, Ig	10	11	35	44	40	46	46	67	468	751	526	404	16+	16+	404	16+						
5	26	M	P, Ig	12	14	27	39	45	42	49	273	668	474	386	14+	14+	386	14+							
6	58	F	P, Ig	14	12	74	62	51	27	28	48	63	87	9	7	9	9	9	7	P†	P	Cy			
7	66	F	P, Ig, S	53	19	24	33	43	41	41	47	59	80	68	92	8+	8+	92	8+	P†					

P indicates prednisone; Ig, intravenous immunoglobulin; DXM, high-dose dexamethasone; S, splenectomy; VC, vitamin C; V, vincristine; aRh, anti-Rh immunoglobulin; Cy, pulse cyclophosphamide; Rtx, rituximab.

*Nadir platelet count before rituximab infusion.

†Daily prednisone doses ranged between 10 mg and 50 mg.

‡This patient refused splenectomy and cytotoxic treatments.

in early responders opsonized B cells block the macrophage system, a mechanism that reminds the mechanism of Fc receptor (FcR) blockade by opsonized red cells following anti-D immunoglobulin treatment.² The decreased production of antiplatelet antibodies accounts for the late and sustained response. In late responders, the FcR blockade effect for some reason does not work, and we only see the late response.

In conclusion, our results confirm that rituximab is an active agent in patients with chronic ITP and that the side effects of this treatment are of mild entity. Contrary to our preliminary observations,¹ responses can be expected not only early during treatment but also up to 6 weeks after the last rituximab infusion. Several issues about this agent, such as the optimum dose and treatment

schedule, the exact mechanisms of action, and long-term side effects, are the objectives of current investigations.

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References

1. Stasi R, Pagano A, Stipa E, Amadori S. Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura. *Blood*. 2001;98:952-957.
2. Bussel JB. Fc receptor blockade and immune thrombocytopenic purpura. *Semin Hematol*. 2000;37:261-266.

To the editor:

Mouse surviving solely on human erythropoietin receptor (EpoR): model of human EpoR-linked disease

Mouse models of human diseases have contributed immensely to our understanding of the function of genes and their mutations and to the disease phenotype. In some cases, the compatibility of human and mouse signal transduction interactions allows replacement of the mouse genes with their human counterparts. In those cases, disease-causing mutations from patients can be introduced into the mouse germ line allowing direct testing of the gene defect *in vivo*. Different genetic approaches have been used to create animal models of human diseases caused by gain-of-function mutations. Human transgenes have been bred onto its mouse homologue knock-out background,¹ or the human gene has been used to directly replace its murine homologue (ie, knocked-in into the mouse gene locus).² A necessary assumption of a functional mutant human gene in the mouse environment is the full compatibility of these genes in both species.

Using the transgenic approach, Yu et al³ have recently shown that the human erythropoietin receptor (EpoR) can rescue erythropoiesis and all other developmental defects associated with the mouse EpoR deficiency. The 80-kb human EpoR transgene recapitulated EpoR expression not only in hematopoietic tissues but also in other tissues known to express EpoR. The human EpoR-rescued mice exhibited normal hematologic parameters (including hematocrit) and normal numbers of erythroid progenitors in the bone marrow. However, Yu et al³ also concluded that mouse erythropoietin (Epo) and human EpoR are fully compatible *in vivo* and questioned earlier observations of others that murine Epo has reduced activity on human cells.⁴

We think that this conclusion is not warranted and provide the following evidence. We replaced the mouse EpoR gene with its human wild-type and mutant homologue in murine embryonic stem (ES) cells.² Animals homozygous for the human wild-type human EpoR gene were slightly, but significantly, anemic compared to their littermates (average hematocrit, 45% vs 49%). Additionally, the animals homozygous for the human wild-type EpoR had lower levels of early erythroid progenitors in the bone marrow (in average 12 vs 19 erythroid colonies per 10⁵ cells plated as assessed in semisolid media containing Epo) and smaller spleens before and after phenylhydrazine injection. These data are either compatible with a lower efficiency of *in vivo* interaction of mouse Epo with human EpoR as suggested by Nicolini et al⁴ or may be explained by

a possible lower efficiency of interaction of the human EpoR with the mouse downstream signaling molecules. Nevertheless, these data prove that mouse and human Epo/EpoR signaling are functionally compatible but have quantitative differences in signaling molecule interactions.

The mutant human EpoR that we knocked-in into the mouse EpoR locus was cloned from a patient with primary familial and congenital polycythemia (PFCP).⁵ We have shown that mice heterozygous or homozygous for the truncated gain-of-function human EpoR were polycythemic and their erythroid progenitors had increased *in vitro* sensitivity to both mouse and human Epo.² Interestingly, another animal model of truncated EpoR without apparent polycythemic phenotype in adults and with comparable *in vitro* responses of wild-type and mutant erythroid progenitors to Epo was later published by Zang et al.⁶ The reason for this discrepancy is not clear; in both cases the resulting truncated EpoR contained one tyrosine. However, it is possible that the mutant

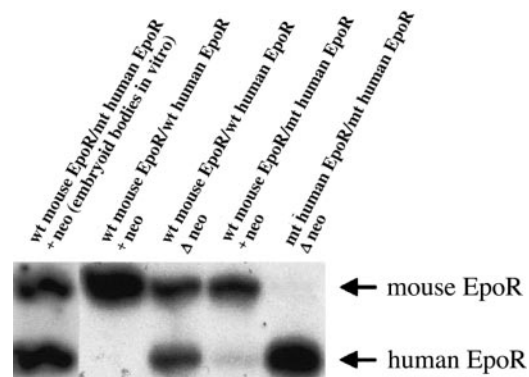


Figure 1. Expression of the human EpoR in the mouse EpoR locus. Primer extension of reverse transcriptase–polymerase chain reaction (RT-PCR) product was used to evaluate relative levels of mouse and human EpoR mRNAs, as described.² The genotypes of ES cells (first lane) or mice (all other lanes) are indicated. The ES cells in the first lane were *in vitro*-differentiated into embryoid bodies in semisolid media with Epo.⁷ The cultures were harvested at day 9 of differentiation, and the cells were used as a template for RT-PCR. The presence of a *neo*-selectable marker did not suppress human EpoR gene expression in the mouse EpoR locus *in vitro*. In the other lanes, bone marrow cells were used as a template for RT-PCR. The retention of a *neo* cassette in the human EpoR gene generated a null or hypomorphic allele *in vivo*. The presence (+ *neo*) or absence (Δ *neo*) of this selectable marker gene is displayed. wt indicates wild type; mt, mutant.



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