

circulating cytokines such as interleukin-1 and tumor necrosis factor- α , which increase endothelial expression of E-selectin. These molecules tether rolling neutrophils by binding to granulocyte Lewis x sialylated carbohydrate (CD15). This allows neutrophil integrins such as complement receptor 3 (CD11b) to adhere to the endothelium via interaction with its ligand intercellular adhesion molecule-1.⁷ The importance of CD64 (Fc-gamma receptor I) as a marker of endothelial adherence in patients with sickle cell disease and its notable increase during sickle cell crisis has been demonstrated. CD11b and CD64 expression on neutrophils is enhanced by G-CSF,^{8,9} providing further insight into mechanisms whereby G-CSF can enhance the trapping of neutrophils in the microcirculation, resulting in vascular occlusion, increased red cell transit time, and sickle cell polymer formation. In addition, sickle cells appear to be more adherent to neutrophils than to normal red cells.¹⁰ Sickle cells also increase neutrophil oxidative activity, which may be important in neutrophil-induced tissue damage during vaso-occlusive episodes.

G-CSF, both by increasing the number of circulating neutrophils and enhancing neutrophil activation and endothelial attachment, may serve to transform a relatively stable steady state into a catastrophic cascade of events resulting in a sickle cell crisis and, in severe cases, multiorgan dysfunction. G-CSF should be given with extreme caution in patients with sickle cell disease. Further study is required to delineate the critical Hb S level that predisposes patients to developing G-CSF-induced sickle cell complications.

Andrew Wei and Andrew Grigg

Correspondence: Andrew P. Grigg, Department of Haematology, Royal Melbourne Hospital, Grattan Street, Victoria, 3050, Australia

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To the editor:

Alloimmunization in Hong Kong southern Chinese transfusion-dependent thalassemia patients

We read with interest Singer et al's report showing an increased alloimmunization and erythrocyte autoimmunization in a group of 64 transfusion-dependent thalassemia patients of predominantly Asian descent.¹ These patients have been receiving phenotypically different red blood cells from predominantly white donors. We would like to share our experience in Hong Kong.

We have been treating a comparable number (68) of thalassemic patients (mean age, 18 years; range, 4-27 years) on regular transfusion. In this group 67 are of southern Chinese origin, and our blood donors are predominantly of the same ethnic origin. Of the 68 patients, 65 had β thalassemia major and 3 had HbE/ β thalassemia heterozygosity syndrome. Using standard blood bank methods, serum was analyzed prior to each transfusion for detection of new antibodies to red blood cell antigens. We employed the same logarithm as Singer's for a further confirmation assay once antibody screening became positive. We always issue fully phenotypically matched blood to those who have an alloantibody in order to prevent further alloimmunization. We rarely need to transfuse K antigen-matched blood, contrary to what Singer et al had suggested.¹ We had observed a total of 9 alloantibodies in 5 patients (of 68) with 7.4% detection rate (unpublished data, April 2001) as compared with 22% (14 of 64) as reported by Singer et al. Only 1 of our 68 patients had an autoantibody, compared with 6 of 64 in Singer et al's report.¹ All our patients with alloantibodies have more than 15 years of regular blood transfusion (range, 15-26

years). Among the 9 alloantibodies detected in our patients (3 Anti-E; 3 Anti-Mi; 1 Anti-HLA; 1 Anti-BG, 1 Anti-BW22), only Anti-E has been reported to be clinically significant, causing a hemolytic transfusion reaction. Singer et al also noticed a relatively high rate of Anti-E (21%), and they could not attribute it to a recipient-donor antigenic discrepancy. Anti-K was not encountered in our patients, unlike the 6 of 19 (31%) anti-K alloantibodies in Singer et al's group. No single alloantibody for c, S, and Fyb was found in our patients. All of our patients' alloantibodies were developed before we introduced universal leukodepletion for thalassemic patients. This observation is in line with Singer et al's observation that a significantly lower alloantibody rate had resulted from the introduction of leukodepletion.¹ Twelve of our patients underwent splenectomy, but only 1 of those 12 had alloantibodies and she developed the antibody before splenectomy. And only 1 of the 12 had autoantibodies after splenectomy. Our experience did not substantiate Singer et al's observation that patients who underwent splenectomy had a higher alloimmunization rate.¹

Previous data on a presumed homogenous population in Greece and Italy also showed an overall low rate (5% to 10%) of alloimmunization.²⁻³ The difference between our experience of lower rate of alloimmunization (7.4%) and Singer et al's higher rate (22%) can be explained by our access to phenotypically matched donors in Hong Kong. We agree with Singer et al's recommendation that the recruitment of Asian blood donors in North America,

just like the recruitment of black donors for sickle cell disease patients, can increase the availability of compatible blood for thalassemia patients, who have a lifelong need for transfusions.

Hok-Kung Ho, Shau-Yin Ha, Chun-Kit Lam, Godfrey C. F. Chan, Tsz-Leung Lee, Alan K. S. Chiang, and Yu-Lung Lau

Correspondence: Hok-Kung Ho, Division of Hematology, Oncology, and Immunology Department of Paediatrics, Queen Mary Hospital, The University of Hong Kong, Hong Kong SAR, China

Response:

Differences in alloantibody types detected in transfused Asian thalassemia patients

Ho et al add important data for better understanding the causes for alloimmunization in Asian thalassemia patients undergoing transfusion. Their data assists in delineating these causes among homogenous and less homogenous donor-recipient populations. The differences in the type of antibodies detected in our and Ho et al's study may also be largely due to the red blood cell phenotypic discrepancy.

We agree with Ho et al that the effect of absence of spleen on allo- and autoantibody development is not completely clear. Our

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data suggested a higher frequency among patients in which the spleen was removed but has not reached statistical significance. Larger studies, in patients from various ethnic backgrounds, are needed to address this issue.

Sylvia T. Singer

Correspondence: Hematology/Oncology, Children's Hospital Oakland, 747 52nd St, Oakland, CA 94609

To the editor:

X chromosome inactivation ratios in female carriers of X-linked sideroblastic anemia

In heterozygous females, an unbalanced X chromosome inactivation pattern (skewed lyonization) may cause disease expression of X-linked disorders,¹ for example, X-linked sideroblastic anemia (XLSA).^{2,3} X chromosome inactivation analyses such as the polymerase chain reaction (PCR)-based human androgen receptor assay (HUMARA) can reveal whether a female has a balanced or a skewed lyonization. Skewing itself can be constitutional or acquired for many different reasons.¹ Moreover, in female carriers of X-linked disorders, skewed lyonization can be fortunate or unfortunate.¹ The latter means a predominant inactivation of the X chromosome harboring the wild-type allele. But the distinction between skewed and balanced lyonization depends on various arbitrary definitions as well as certain technical variables.

Cazzola et al⁴ recently reported an Italian family with females heterozygous for an *ALAS2* mutation that may cause XLSA. The degree of lyonization in these individuals was determined in leukocytes by the cleavage ratio between alleles from the HUMARA. For this assay, the methods for the detection and semiquantitative assessment of PCR products have crucial importance. Cazzola et al used silver-stained nondenaturing polyacrylamide gels and densitometric scanning for determination of cleavage ratios and did not provide methods for their calculation or correction. For semiquantitation, we recommend the use of an automated laser fluorescence sequencer or a similar device for enhanced resolution.⁵

Cazzola et al, like some other authors, attribute "excessive skewing"^{4(p4364)} to allele ratios higher than 3.0 while allele ratios below 3 are defined as balanced. Elsewhere, a ratio between 1.85

and 4.0, as found in the 3 female carriers, has been termed "moderately skewed,"^{6(p32)} and several other authors considered an "extreme lyonization" or "monoclonality" only when allele ratios were above 10.^{7(p62),8(p1581)} The allele ratio can also be translated into the percentage of inactivated X chromosomes harboring the wild-type allele as follows: $[\text{ratio} / (\text{ratio} + 1)] \times 100$.⁹ Thus, for case II-2 in Cazzola et al's report, the ratio of 3.2 would translate into 76% of cells with an inactive wild-type *ALAS2* allele. But sequence analysis of cDNA derived from her reticulocyte RNA showed only expression of the wild-type allele. This finding is not discussed and is difficult to reconcile. Theoretically, a distinct erythroid lineage-specific X chromosome inactivation pattern (XCIP) due to a postinactivation selection may provide a possible explanation and could be resolved by X chromosome inactivation analysis of erythroid precursors.

Unfortunately, the only anemic person in the family reported by Cazzola et al (the proband; hemoglobin level 5.2 g/dL) was not informative, but an extremely skewed lyonization, for example, 99% of cells having an inactive wild-type *ALAS2* allele, can be assumed. Using the above formula in cases II-3 and III-2 (with HUMARA cleavage ratios of 4.0), 80% of the cells should have an inactive wild-type *ALAS2* allele. It is puzzling how 20% of cells with an active wild-type *ALAS2* allele can account for the lack of anemia in these individuals.

Finally, the authors postulate familial skewing. But the moderately skewed XCIP in the 3 females' leukocytes could also be the result of an age-related stochastic event, as occurs in approximately