BMC Biotechnol. 2017;17(1):61.
4. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet. 2009;373(9674):1550-1561.
5. Warren EH, Matsen FA 4th, Chou J. High-throughput sequencing of B- and T-lymphocyte antigen receptors in hematology. Blood. 2013;122(1):19-22.
6. Padovan E, Casorati G, Dellabona P, Meyer S, Brockhaus M, Lanzavecchia A. Expression of two T cell receptor alpha chains: dual receptor T cells. Science. 1993;262(5132):422-424.
7. Wang C, Sanders CM, Yang Q, et al. High throughput sequencing reveals a complex pattern of dynamic interrelationships among human T cell subsets. Proc Natl Acad Sci U S A. 2010;107(4):1518-1523.
8. Ni PP, Solomon B, Hsieh CS, Allen PM, Morris GP. The ability to rearrange dual TCRs enhances positive selection, leading to increased Allo- and Autoreactive T cell repertoires. J Immunol. 2014;193(4):1778-1786.
9. Morris GP, Uy GL, Donermeyer D, Dipersio JF, Allen PM. Dual receptor T cells mediate pathologic alloreactivity in patients with acute graft-versus-host disease. Sci Transl Med. 2013;5(188):188ra174.
10. Balakrishnan A, Gloude N, Sasik R, Ball ED, Morris GP. Proinflammatory Dual Receptor T Cells in Chronic Graft-versus-Host Disease. Biol Blood Marrow Transplant. 2017;23(11):1852-1860.
11. Attaf M, Huseby E, Sewell AK. alphabeta T cell receptors as predictors of health and disease. Cell Mol Immunol. 2015;12(4):391-399.
12. Zvyagin IV, Mamedov IZ, Tatarinova OV, et al. Tracking T-cell immune reconstitution after TCRalphabeta/CD19-depleted hematopoietic cells transplantation in children. Leukemia. 2017;31(5):1145-1153.
13. Kanakry CG, Coffey DG, Towlerton AM, et al. Origin and evolution of the T cell repertoire after posttransplantation cyclophosphamide. JCI Insight. 2016;1(5):e86252.
14. Bleakley M, Heimfeld S, Loeb KR, et al. Outcomes of acute leukemia patients transplanted with naive T cell-depleted stem cell grafts. J Clin Invest. 2015;125(7):2677-2689.
15. Gkazi AS, Margetts BK, Attenborough T, et al. Clinical T Cell Receptor Repertoire Deep Sequencing and Analysis: An Application to Monitor Immune Reconstitution Following Cord Blood Transplantation. Front Immunol. 2018;9:2547.
16. Horowitz M, Schreiber H, Elder A, et al. Epidemiology and biology of relapse after stem cell transplantation. Bone Marrow Transplant. 2018;53(11):1379-1389.
17. Negrin RS. Graft-versus-host disease versus graft-versus-leukemia. Hematology Am Soc Hematol Educ Program. 2015;2015:225-230.
18. Fleischhauer K, Shaw BE. HLA-DP in unrelated hematopoietic cell transplantation revisited: challenges and opportunities. Blood. 2017;130(9):1089-1096.
19. Arrieta-Bolanos E, Crivello P, Metzing M, et al. Alloreactive T Cell Receptor Diversity against Structurally Similar or Dissimilar HLA-DP Antigens Assessed by Deep Sequencing. Front Immunol. 2018;9:280.
20. Howie B, Sherwood AM, Berkebile AD, et al. High-throughput pairing of T cell receptor alpha and beta sequences. Sci Transl Med. 2015;7(301):301ra131.

## Mosaicism by somatic non-functional mutations: one cell lineage at a time

**Willy Albert Flegel**[1,2]

*[1]Department of Transfusion Medicine, NIH Clinical Center, National Institutes of Health, Bethesda, MD and [2]Department of Pathology, Georgetown University Medical Center, Washington, DC, USA*

*E-mail: WILLY ALBERT FLEGEL - waf@nih.gov*

*doi:10.3324/haematol.2018.208165*

Somatic mutations are abundant in most cells of our tissues.[1,2] The impact of any somatic mutation may be small and temporary if it occurs in differentiated cells without giving rise to malignant growth by unlocking their terminal differentiation. We expect a much wider and lasting impact from the same somatic mutations if they occur during earlier steps of differentiation. If they arise in a stem or progenitor cell, a whole set of cells (the lineage downstream of the progenitor) will be affected. Any such novel somatic mutation may stay with the individual for life, when different somatic mutations can accumulate in cell lineages over time.[3] The combination of somatic mutations becomes not only part of our lives, but contributes to our phenotype.[4] The variety of somatic mosaicism that will develop differs randomly among individuals and forms the basis of our healthy constitutions as well as of some medical conditions.

In this issue of the Journal, Dauber *et al.* present a technically advanced study[5] describing the molecular causes of mosaicism in 2 patients. The antigens of the Rhesus blood group system (ISBT 004) served as markers allowing the authors to take advantage of routine serology and detect affected individuals. The patients with red cell mosaicism were identified by the loss of the c antigen (RH4) in a subset of their red cells. The causes of this serological phenotype were traced to distinct precursor stages of myeloid and pluripotent stem cells, respectively.[5] As the antigens were only the markers and not the focus, this study, at the intersection of 'erythropoiesis gone wrong' and red cell antigens, has relevance beyond blood groups and offers valuable information to the hematology community.

Red cell mosaicism was documented in leukemia for ABO antigens in 1957,[6,7] and for Rh a few years later;[8] it has been documented for various blood groups many times since then.[5,9,10] During routine blood group typing worldwide, immunohematologists repeatedly encounter the incidental finding of a 'mixed field' agglutination or a discrepancy with previous results in the patient's health record. In both patients of the current study,[5] 'mixed field' agglutination was observed and prompted further investigation. Barring technical issues with the serology, such findings are infrequent, although not rare, in the absence of transfusions. Many patients could be identified with mosaicism that cannot be explained simply by recent transfusion, and these patients could then be followed up in order to evaluate the clinical implications. Possible causes are loss of heterozygosity (LOH) associated with loss of tumor suppressor gene functions,[11] or copy-neutral LOH associated with the gain of oncogenic mutations.[12,13]

Despite the discrepancy in blood group, the immediate transfusion support given to these patients is usually straightforward. Hence, further analysis is not considered necessary within the practical approach to clinical care. The clinical prognosis of LOH in hematologically asymptomatic patients is currently unknown, although the authors point out that this may now be changing. The diagnosis of red

cell mosaicism could be important for the patients, who are not regularly informed of the incidental finding. Because the technologies required to confirm mosaicism lie far outside routine procedures, they are not available to clinical laboratories because of the limited resources available. This was not the case for these 2 patients[5] whose pathophysiology was examined in unusual detail.

The current study[5] represents a step forward in this field, as the exact types of precursors, affected by distinct somatic mutations, have been determined. LOH was, indeed, the underlying cause in both patients, affecting the *RH* locus on the short arm of chromosome 1 and encompassing at least 26.7 and 42.4 Mb, respectively. Such large deletions of parts of a chromosome are considered rare in patients without malignancies or other hematologic pathologies. The *RHCE* gene spans only 60 kb and an *RHCE* deletion is still extremely rare in genomic DNA. As both large deletions encompassed the *RHD/RHCE* gene loci, they explained the serological phenotype with the loss of the c antigen in approximately 50% of the patients' red cells (Figure 1).

Besides the deletion of a chromosomal segment or a whole gene, less intrusive mechanisms, all the way down to 1 single nucleotide polymorphism (SNP), can functionally mimic the same LOH for *RHCE*, which prompted the authors to study these 2 patients. Like any DNA variation, somatic mutations,[14] if found in exons, can affect the protein by missense and non-functional mutations, and less so by silent mutations. A novel SNP in a somatic cell lineage occurs much more frequently than any huge alteration affecting long DNA segments,[5] but can be as important for the blood group phenotype.[15] A SNP may also have an impact on gene regulation when occurring in the introns, the 3'- or 5'-untranslated regions of a gene, or somewhere in the long DNA segments interspersed between genes. Genes differ in their propensity to lose their function,[16] as do the mechanism causing changes, ranging from an SNP in an *RH* gene,[17] to large deletions affecting the *RH* gene locus[18,19] and adjacent genes.[5,13]

Somatic mosaicism is always acquired and occurs during mitosis in 1 cell lineage derived from 1 zygote, often after

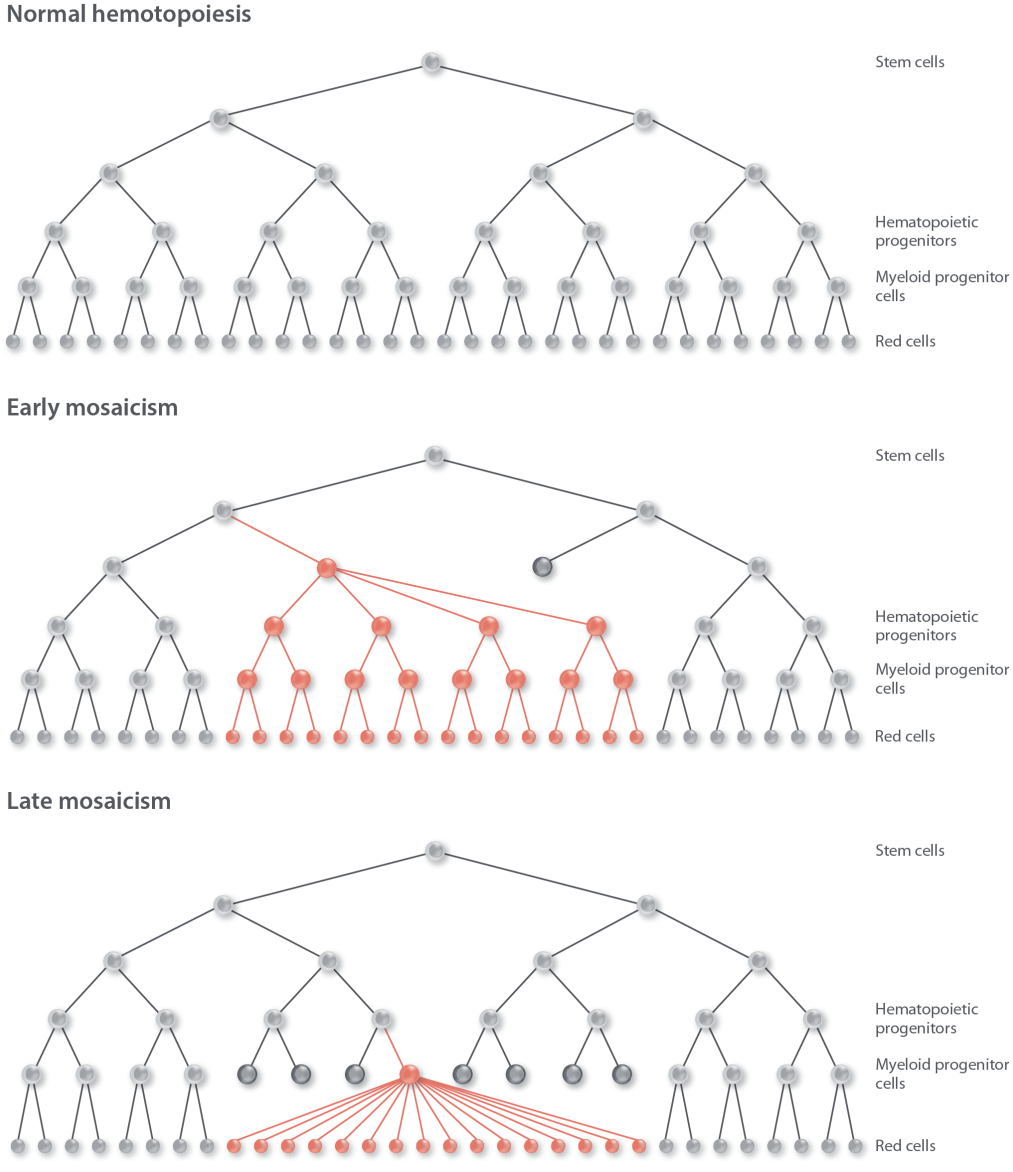


**Figure 1. Schematic representation of differentiation from early stem to red cells.** A model for normal hematopoiesis (top) assumes equal cell division at all stages (lines with open circles). In the models for early (middle) and late (bottom) mosaicism, the red cells eventually reach 50% clonality. Some cell lineages are squeezed out, possibly experiencing apoptosis (closed circles) before differentiating into red cells. This 50% mosaicism represents the red cell populations actually observed in the peripheral blood of the 2 patients in the study by Dauber *et al.*[5] reported in this issue of the Journal.

many cell divisions during aging. Most acquired chimerism is iatrogenic and takes place after allogeneic solid organ or hematopoietic progenitor cell transplantations. Diagnostic laboratories routinely come across such patients when testing peripheral blood, and hematologists and other consultants should be willing to share this clinically quite important patient history with the laboratory. In comparison, congenital chimerism, as sometimes found by blood group typing,[19] is much less common. The diagnostic hallmark for chimerism, whether congenital or acquired, are 2 entirely different genotypes derived from 2 zygotes and found throughout the genome in 2 sets of cells or tissues. In contrast, a typical somatic mosaicism is limited to only 1 chromosomal location.

In depth genotyping at the single cell level may eventually reveal a host of mosaicisms in individuals and even in a set of cells that is presumed to belong to 1 cell lineage. Certain congenital diseases predispose to somatic mutations, such as in fragile X syndrome. Conversely, the highly individual accumulation of somatic mutations causes the great variety of phenotype in dyskeratosis congenita. The exact combination of mosaicism accrued throughout life in a variety of cell lineages constitutes the substrate of single cell medicine. Current progress in gene editing will allow somatic mutations in differentiating cells to be modified or corrected; this will raise few ethical concerns since the germline is not affected.

It was possible to clarify more molecular details and the regulatory mechanism was addressed in both of these patients. The exact break points involved in the deletion events, as defined by nucleotide sequence, can be determined with current technology. This would also allow the claim that the proposed *CDe* haplotype is duplicated in the affected cell lineages to be corroborated. While the patterns may seem random, close inspection can reveal preferred sites. The nucleotide sequences in the break point regions often hint at distinct deletion and duplication events[18,20,21] involving specific molecular repair mechanisms.

Variants of the RhCE protein are not known to exert any regulatory effect or growth advantage, and one or more of the many other genes located in the deleted or duplicated long DNA segments could be evaluated as a cause for the clonal expansion. Red cells represent 84% of all human cells.[22] Isn't it an odd coincidence that both patients happen to have approximately half of their red cells affected despite 2 distinct LOH mutations (Figure 1)? While the single pluripotent stem cell and myeloid precursor, having incurred different LOH mutations,[5] can develop some clonality,[23] they are hardly expected to produce 50% of all red cells in each patient. It might be an observation bias that these reported findings are so similar, if many clonal events of a lesser degree are overlooked in routine clinical practice.

With the advent of mass scale sequencing of whole genomes in cell lines and cell lineages, mosaicism is becoming more frequently observed. And it can only become more important as single cell genomics is used to study aging populations. As understanding of underlying mechanisms widens, we will observe similar mutation patterns and their prevalence. Each cell lineage will accumulate its distinct combination of mutations, and these are bound to vary among individuals; even between monozygotic, identical twins. As 99% of all inherited nucleotide sequence in any individual is homozygous, the acquired heterozygous sequence, along with the common inherited status, may constitute a major cause for phenotypes, and ultimately diseases. Today, since systematic analysis of mosaicism remains challenging, its role as a critical mechanism for disease and aging may quite possibly be underappreciated.[4]

***Acknowledgments***

*The author thanks Harvey Gordon Klein for his review. Supported by the Intramural Research Program (project ID Z99 CL999999) of the NIH Clinical Center.*

## References

1. Yoshizato T, Dumitriu B, Hosokawa K, et al. Somatic mutations and clonal hematopoiesis in aplastic anemia. N Engl J Med. 2015;373(1):35-47.
2. Babushok DV, Duke JL, Xie HM, et al. Somatic HLA mutations expose the role of class I-mediated autoimmunity in aplastic anemia and its clonal complications. Blood Adv. 2017;1(22):1900-1910.
3. Frumkin D, Wasserstrom A, Kaplan S, Feige U, Shapiro E. Genomic variability within an organism exposes its cell lineage tree. PLoS Comput Biol. 2005;1(5):e50.
4. Machiela MJ, Chanock SJ. The ageing genome, clonal mosaicism and chronic disease. Curr Opin Genet Dev. 2017;42:8-13.
5. Dauber EM, Mayr WR, Hustinx H, et al. Somatic mosaicisms of chromosome 1 at two different stages of ontogenetic development detected by Rh blood group discrepancies. Haematologica. 2019;104(3):632-638.
6. van Loghem JJ, Dorfmeier H, van der Hart M. Two A antigens with abnormal serologic properties. Vox Sang. 1957;2(1):16-24.
7. Gold ER, Tovey GH, Benney WE, Lewis FJ. Changes in the group A antigen in a case of leukaemia. Nature. 1959;183(4665):892-893.
8. Tovey GH, Lockyer JW, Tierney RB. Changes in Rh grouping reactions in a case of leukaemia. Vox Sang. 1961;6:628-631.
9. Kolins J, Holland PV, McGinniss MH. Multiple red cell antigen loss in acute granulocytic leukemia. Cancer. 1978;42(5):2248-2253.
10. Kolins J, Allgood JW, Burghardt DC, Klein HG, McGinniss MH. Modifications of B, I, i, and Lewisb antigens in a patient with DiGuglielmo's erythroleukemia. Transfusion. 1980;20(5):574-577.
11. Thiagalingam S, Foy RL, Cheng KH, Lee HJ, Thiagalingam A, Ponte JF. Loss of heterozygosity as a predictor to map tumor suppressor genes in cancer: molecular basis of its occurrence. Curr Opin Oncol. 2002;14(1):65-72.
12. O'Keefe C, McDevitt MA, Maciejewski JP. Copy neutral loss of heterozygosity: a novel chromosomal lesion in myeloid malignancies. Blood. 2010;115(14):2731-2739.
13. Montemayor-Garcia C, Coward R, Albitar M, et al. Acquired RhD mosaicism identifies fibrotic transformation of thrombopoietin receptor-mutated essential thrombocythemia. Transfusion. 2017;57(9):2136-2139.
14. Miao X, Li X, Wang L, Zheng C, Cai J. DSMNC: a database of somatic mutations in normal cells. Nucleic Acids Res. 2018 Oct 31. [Epub ahead of print]
15. Wagner FF, Flegel WA. Polymorphism of the h allele and the population frequency of sporadic nonfunctional alleles. Transfusion. 1997;37(3):284-290.
16. Schmid P, Flegel WA. Codon usage in vertebrates is associated with a low risk of acquiring nonsense mutations. J Transl Med. 2011;9:87.
17. Flegel WA, Eicher NI, Doescher A, et al. In-frame triplet deletions in RHD alter the D antigen phenotype. Transfusion. 2006;46(12):2156-2161.
18. Wagner FF, Flegel WA. RHD gene deletion occurred in the Rhesus box. Blood. 2000;95(12):3662-3668.
19. Wagner FF, Frohmajer A, Flegel WA. RHD positive haplotypes in D negative Europeans. BMC Genet. 2001;2:10.
20. Srivastava K, Stiles DA, Wagner FF, Flegel WA. Two large deletions extending beyond either end of the RHD gene and their red cell phenotypes. J Hum Genet. 2018;63(1):27-35.
21. Wagner FF, Flegel WA. RHCE represents the ancestral RH position, while RHD is the duplicated gene. Blood. 2002;99(6):2272-2273.
22. Sender R, Fuchs S, Milo R. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. Cell. 2016;164(3):337-340.
23. Cooper JN, Young NS. Clonality in context: hematopoietic clones in their marrow environment. Blood. 2017;130(22):2363-2372.