Model of mismatch repair (MMR)–dependent age-related changes in the hematopoietic stem cell pool. At birth, if any, genetic alterations occur in the hematopoietic stem cell (HSC) pool (blue ovals). Over the next 45 years, HSCs accumulate low levels of DNA damage (ie, base-mispairs and/or microsatellite instability [MSI]; red caret) that either escape MMR or are caused by MLH1 loss. After 45 years of age, accumulation of damage accelerates (2 red carets) due to subsequent loss of MLH1 and MMR capacity potentially leading to HSC loss of function (dashed ovals) and/or HSC malignant transformation (green ovals).

Specifically, at birth HSCs and HPCs have fully functional MMR capacities and have few, if any, MSI or point mutations. During the first 45 years of life, a small number of genetic alterations accumulate, but most CFCs retain full MMR capacity. After 45 years, loss of MMR capacity in CFCs is accelerated due to MLH1 hypermethylation resulting in increased genomic instability. It is reasonable to suggest that this accumulation of genetic alterations drives loss of HSC function and/or malignant transformation. The MMR-mediated HSC dysfunction model differs from other genomic instability syndromes where affected cells require adaptive mutations to survive DNA damage. In contrast, loss of MMR-mediated DNA damage surveillance and signaling makes MMR-deficient cells immediately resistant to specific types of DNA damage.

Age-induced MSI may function as an early marker of hematopoietic dysfunction and provides a target for therapeutic intervention. As MMR capacity is lost due to epigenetic regulation of MLH1, strategies to restore MMR function and MLH1 expression could ameliorate age-related hematopoietic defects. An obvious class of drugs would be demethylating agents; however, these drugs affect global methylation patterns, have serious side effects, and the effects of continual treatment are unknown. Another treatment option may be to selectively target cells that are MMR-deficient; however, because a significant proportion of CFCs are MMR-deficient this would most likely deplete stem cell pools and might make things worse. Therefore, strategies are needed to specifically target MLH1 re-expression, which may not only alleviate age-related hematopoietic dysfunction, but other age-related pathologies.

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HLA-DO expands the repertoire of peptides presented by class II MHC molecules such as HLA-DR. (Top) In the MIIC, HLA-DM facilitates the exchange of CLIP for specific DM-resistant peptides into the binding groove of class II molecules, which are then transported to the cell surface. The loading of DM-sensitive peptides is inhibited. (Bottom) In the presence of HLA-DO, both DM-resistant and DM-sensitive antigens are loaded, leading to a more diverse repertoire of peptide/class II MHC molecules displayed on the cell surface. Professional illustration by Debra T. Dartez.

CD4+ T lymphocytes recognize short peptides, generally less than 20 residues long, presented on the cell surface by the “classic” class II MHC molecules HLA-DR, -DQ, and -DP. Although the complex repertoire of peptides presented on class II cells by their complement of class II molecules has not yet been comprehensively defined, it is clear that this repertoire comprises peptides derived from the degradation of both exogenous and endogenous proteins. HLA-DR, -DQ, and -DP molecules are loaded with their peptide cargo in a late endosomal compartment called the MHC class II compartment (MIIC) in a process that is catalyzed by the nonclassic class II molecule HLA-DM, and involves displacement of CLIP, a peptide derived from proteolysis of invariant chain. Although HLA-DM does not itself have peptide-binding activity, it does bind to classic class II molecules such as HLA-DR and in so doing modifies their affinity for specific peptides, thus functioning as a peptide editor. Before exiting the MIIC and moving to the cell surface, HLA-DR, -DQ, and -DP molecules can undergo repetitive cycles of binding to HLA-DM with repeated peptide exchange, leading to a finely honed repertoire of peptides presented on the surface.

The peptide editing activity of HLA-DM in the MIIC can be modified by HLA-DO, another nonclassic class II MHC molecule that likewise has no peptide-binding activity of its own. Although the consensus view of HLA-DO function holds that it primarily inhibits the peptide editing activity of HLA-DM, relatively few published studies have evaluated the effect of HLA-DO expression on the presentation by class II+ cells of specific antigenic peptides, particularly those derived from endogenous proteins. The study from Kremer et al now argues quite convincingly that this traditional view of HLA-DO function is due for thorough re-evaluation. Through precise dissection of the effect of HLA-DM and HLA-DO expression on the presentation of 6 antigenic peptides, all derived from endogenous proteins, which elicited CD4+ T cell responses in vivo, the authors demonstrate that the net effect of HLA-DO expression is to expand the repertoire of peptides presented on the surface by class II MHC. This expanded peptide repertoire, however, is only presented by the limited range of cell types in which HLA-DO is expressed, namely, B lymphocytes, dendritic cells, and thymic epithelial cells (see figure).

Kremer and colleagues had previously isolated 6 alloreactive CD4+ T-cell clones from patients who had undergone allogeneic hematopoietic cell transplantation (HCT) from MHC-matched donors. They subsequently established that these clones recognized peptides derived from endogenous recipient proteins encoded by polymorphic genes, that is, that they recognized minor histocompatibility (H) antigens, and identified the specific class II MHC alleles that presented the 6 peptides for recognition by CD4+ T cells. Using the T-cell clones as sensitive reagents to detect the presence of their cognate antigens on the surface of target cells expressing the appropriate class MHC II restriction molecules, they could evaluate the effect of HLA-DM, HLA-DO, or both, on presentation of the antigenic peptides.

Kremer et al found that expression of the peptide editor HLA-DM in the absence of HLA-DO allowed presentation of 3 of the 6 minor H antigens, which they labeled DM-resistant antigens, but did not permit presentation of the other 3, which they accordingly termed DM-sensitive antigens. Coexpression of HLA-DO with HLA-DM, however, allowed presentation of the 3 DM-sensitive antigens, with negligible effect on the presentation of the 3 DM-resistant antigens. Epitope-exchange experiments assessing the recognition of target cells transduced with genes encoding recombinant proteins in which a DM-resistant peptide had been replaced by a DM-sensitive peptide, as well as the reciprocal manipulation, demonstrated that DM-sensitivity was an intrinsic property of the antigenic peptide and did not depend on the identity of the protein from which it was derived. Mutation of specific residues within the HLA-DR αβ heterodimers with HLA-DM had no effect on the presentation of 2 DM-resistant antigens but enabled the presentation of 2 DM-sensitive antigens, demonstrating that the...
property of DM-sensitivity was dependent on these DM-DRA contact residues.

To determine whether the property of DM-sensitivity was specific to the 3 antigenic peptides the authors had previously characterized, or a more general phenomenon, Kremer et al tested the effect of HLA-DM expression on target cell recognition by random CD4+ T-cell libraries established from peripheral blood of healthy donors. The DM-sensitive phenotype was common among the pools from each of the libraries, suggesting that peptide editing by HLA-DM has an extensive influence on the repertoire of peptides presented by class II MHC.

The results of these studies have potentially broad implications that extend from self-tolerance and autoimmunity to tumor immunology and immunotherapy. The narrow range of cells in which HLA-DO is expressed, and the fact that its expression is poorly, if at all, induced in other cell types by inflammatory stimuli such as interferon-γ, suggest that DM-sensitive antigens will likewise show expression that is limited to a very narrow range of cell types. Kremer et al point out that DM-sensitive antigens expressed in malignant cells that express HLA-DO represent attractive targets for immunotherapy, even under inflammatory conditions where class II MHC expression is likely to be induced in many different tissues. Could the exquisitely tissue-specific expression of HLA-DO be exploited, for example, to enhance graft-versus-leukemia effects after allogeneic HCT without inducing graft-versus-host disease? The results of Kremer et al provide us with a promising path to explore.

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**Comment on Visconte et al**, page 3173

**SF3B1 and the riddle of the ring sideroblast**

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In this issue of *Blood*, Visconte and colleagues report on their investigations into the pathophysiologic effects of altered SF3B1 in patients with myelodysplastic syndromes (MDS).1 A crucial component of the spliceosomal U2 snRNP complex, SF3B1 participates in normal RNA splicing. It was a surprise when whole genome sequencing studies showed that the most frequent recurrent mutations in MDS affect the spliceosome machinery. Collectively, spliceosome mutations are found in approximately 45% of all MDS patients. The strongest genotype-phenotype correlation exists between mutations of SF3B1 and the sideroblastic phenotype, which is caused by iron accumulation in erythroblast mitochondria. SF3B1 mutations are found in 60%–80% of patients with refractory anemia with ring sideroblasts (RARS) or RARS with thrombocytosis (RARS-T).2,3 Visconte et al found that patients with SF3B1 mutations have particularly coarse mitochondrial iron deposits.3 Knockdown experiments in K562 cells had an impact on intron-splicing, but did not produce RARS, probably because K562 cells are not sufficiently “erythroid.” However, meyamycin, a pharmacologic SF3B inhibitor, induced the formation of RARS in healthy bone marrow cells, corroborating the view that insufficiency of SF3B1 is important. Finally, the causative role of SF3B1 haploinsufficiency was confirmed in SF3B1 heterozygous mice showing RARS in the bone marrow.

RNA sequencing analysis of SF3B1 mutants yielded many exons differentially used in the SF3B1 mutant relative to normal groups, but none of them were obvious troublemakers for mitochondrial iron metabolism. In contrast to Papaemmanuil et al,1 Visconte et al did not find underexpression of key biologic pathways involved in mitochondrial function. It is also puzzling that they did not find any difference in exon-usaging or gene expression of ABCB7, considering that Boulwood et al showed substantial down-regulation of ABCB7 expression in patients with RARS.4 A possible explanation is that Visconte and colleagues1 examined total bone marrow cells, while CD34+ cells were studied by the other investigators.3,4 Until these discrepancies are resolved, we cannot be sure that SF3B1 mutations interfere with mitochondrial iron metabolism through pathologic splicing and altered gene expression.

Other explanations should be considered. The absence of frameshift, nonsense, and splice-site mutations, the lack of key structural amino acid residues as sites for mutation, and the fact that the mutations are less deleterious than expected on the basis of chance all suggest that the mutated SF3B1 protein is likely to retain structural integrity, albeit with presumably altered function.3 Such altered function may either compromise a hitherto unknown physiologic role of SF3B1 in mitochondrial iron metabolism, or create a novel, unphysiologic effect on mitochondrial iron handling. SF3B1 does have functions unrelated to the splicing complex. For instance, its physical interaction with class II polycomb group proteins is needed for PeG-mediated repression of Hox genes, and skeletal abnormalities in SF3B1−/− mice appear to be independent of the alteration of general gene expression.5 Therefore, mutant SF3B1 may have pathophysiologic effects because of abnormal protein–protein interactions that have nothing to do with the splicing apparatus.
Diversifying the MHC peptide portfolio

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