Because the risk of CLL associated with the *POT1* p.Gln376Arg mutation was increased 3.61-fold, it suggests that shelterin gene mutations have moderate penetrance. Such an assertion is supported by the observation that the unmutated allele is not lost in the CLL cells of mutated carriers. Determination of the true penetrance of these variants will likely require the study of the risk loci in unaffected individuals, for example, the siblings of the affected patients, although ethical considerations may limit this analysis.

The understanding of the role of genetic alterations in the pathogenesis of CLL has been expanded in the last few years with extensive genome wide analysis focusing on 2 major aspects. Whole genome/exome sequencing efforts have elucidated the landscape of somatic mutations in untreated CLL patients, with the identification of ~80 highly confident driver genes (see figure).[7,8] On the other hand, genome-wide association studies (GWASs) have identified 31 susceptibility loci conferring an increased risk of developing the disease (see figure and results of 8 comprehensive GWASs referred to by Speedy et al). Some of these loci are within or in the vicinity of genes whose function may influence the development of the disease, but most of them are in noncoding regions, and their direct role in the pathogenesis of CLL is not understood. Most genes identified in both types of studies are different, and only *POT1, BCL2,* and *IRF4* are found in both subsets of genes (see figure). The small group of genes in the overlap between the 2 subsets is intriguing. Possible reasons for this minor overlap are that genes related to the respective susceptibility loci and somatic mutated genes may act at different moments in the development of the disease. On the other hand, although the genes in both subsets are different, they may be targeting similar pathways. For example, germ line variations related to *TERT* interfere with telomeric function, which are also targeted by somatic mutations in *POT1*, frequently found in CLL.

A challenge for the future will be to detect underlying germ line alterations in the other CLL families studied for which thus far no germ line mutations have been detected. Potentially, germ line mutations may be found in noncoding regions. Intriguingly, it is becoming clear that noncoding regions in the genome may affect gene expression by interaction with their target genes in 3-dimensional (3D) space in the nucleus.[9] Consequently, genetic alterations within noncoding regions may affect distant target genes. This was shown for somatic mutations in a distant *PAX5* enhancer that significantly reduced the expression of this master B-cell regulator in affected CLL cases.[7] Similarly, the functional and 3D analysis of the CLL susceptibility locus located in an enhancer at 15q15.1 was identified to target the antiapoptotic BCL2 pathway, by influencing the expression of BMF in CLL.[10] Hence, an integrative analysis of genetic screens together with gene expression and comprehensive epigenetic studies would be instrumental to better understand the role of susceptibility loci and somatic mutations in noncoding regions in the pathogenesis of CLL.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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DOI 10.1182/blood-2016-09-735688

● ● ● PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Uhl et al, page 2327

The older the faster: aged neutrophils in inflammation

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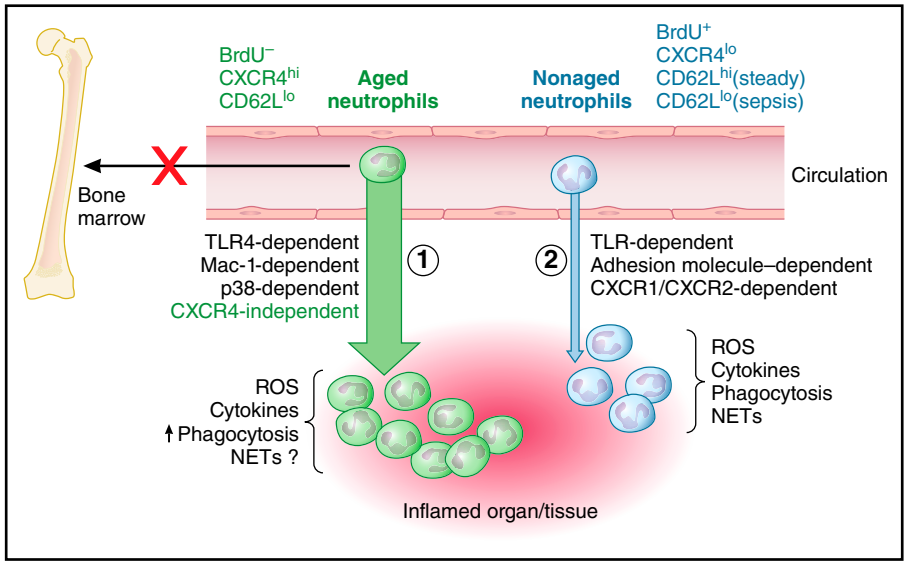
In this issue of *Blood*, Uhl et al provide evidence that aged neutrophils arrive at sites of inflammation prior to nonaged neutrophils, dominate the inflammatory focus in terms of numbers, and phagocytize bacteria more efficiently.[1]

Until now, aged neutrophils were considered end-stage cells whose fate was limited to disposal in specialized organs. Moreover, it was believed that aged neutrophils might migrate poorly to sites of inflammation as their expression of CXCR2, the receptor for several major neutrophil-targeted chemokines, such as CXCL8/interleukin-8, is decreased.[2] The discovery that these "age-wise" cells are the first and dominant subtype of neutrophil to be recruited to the sites of infection sheds new light on efficiency and adaptability of the immune system.

Although the life span of neutrophils in circulation in vivo might be somewhat longer than was commonly believed,[3] their half-life can still be expressed in hours rather than days. In the absence of infection, where neutrophils are recruited to and die within inflammatory sites, neutrophils in circulation do not die in the bloodstream but rather are eliminated in bone marrow (predominant site), spleen, or liver by specialized macrophages.[2] Recently, it has been shown that the aging of neutrophils is microbiota-driven and depends on Toll-like receptors (TLRs), including TLR-2 and TLR-4,



Dynamics and phenotypes of aged and nonaged neutrophils during acute systemic inflammation. In response to lipopolysaccharide (LPS), aged neutrophils (1) arrive first to the inflamed organs/tissues and are followed by nonaged neutrophils (2). Simultaneously, the homing of aged neutrophils to bone marrow is altered and limiting their clearance. At the inflammatory site, aged neutrophils phagocytosize more intensively than the nonaged cells but reactive oxygen species (ROS) production and cytokine release are the same as in nonaged neutrophils. Recruitment of aged neutrophils is TLR-4 and macrophage-1 antigen (Mac-1)-dependent, however, the chemokine receptor CXCR4 is not involved in this process. 5-Bromo-2′-deoxyuridine (BrdU) is not incorporated into postmitotic cells, thus aged neutrophils are $BrdU^-$ giving them an overall phenotype of $BrdU^-CXCR4^{hi}CD62L^{low}$ whereas nonaged neutrophils are $BrdU^+CXCR4^{low}CD62L^{hi}$ under steady-state conditions. ?, Not studied yet in this model. Professional illustration by Patrick Lane, ScEYEnce Studios.

in a Myd88-dependent manner.[4] Uhl et al extend this observation by reporting that these TLRs (TLR-4 in particular) are also critical for recruitment of aged neutrophils to the inflammatory focus, and this recruitment process depends on a p38 MAPK pathway (see figure).

For many years, it has been recognized that aged neutrophils acquire higher expression of CXCR4, a receptor that helps to redirect these cells to bone marrow, a tissue that constitutively produces its ligand, CXCL12/stromal cell-derived factor 1 (SDF-1). Furthermore, aged neutrophils have lower expression of selectin L (CD62L), a molecule that is used during first steps of the transmigration cascade to the inflamed tissues.[1] This marker, however, cannot be used to discriminate aged and nonaged neutrophils during endotoxemia as nonaged neutrophils lose much of their CD62L signal upon LPS challenge, most probably due to the L-selectin shedding, a phenomenon commonly observed in sepsis.[5] Interestingly, despite higher expression of CXCR4, the redirection of aged neutrophils to bone marrow is halted during endotoxemia. It still remains to be resolved what chemokines/chemokine receptors are responsible for the recruitment of the aged neutrophils to the inflammatory sites. As the expression of CXCR1 is unaltered between the 2 populations, aged and not-aged,[6] this receptor may be an important candidate molecule mediating recruitment of aged neutrophils. Furthermore, it cannot be excluded that ligands of CXCR1, or the other putative chemokine receptors operating in this system, desensitize CXCR4, preventing cell responsiveness to SDF-1 signal and homing to bone marrow.

Leukocyte recruitment depends on presence of adhesion molecules, and in the case of integrins, on their affinity for respective ligands. Increased expression of numerous adhesion molecules, including lymphocyte function–associated antigen-1 (CD11a/β2), Mac-1 (CD11b/β2), and their ligand ICAM-1, was previously described on aged neutrophils[4] but Uhl et al report that Mac-1 expression is further increased during LPS-induced inflammation. This molecule is primarily involved in events leading to adherence and crawling of neutrophils during diapedesis. Importantly, Uhl et al showed that ICAM-1 more strongly associated with aged neutrophils and β2 integrins on the aged neutrophil existed in their higher affinity confirmation, allowing for better association with ICAM-1. These findings might provide an explanation for the stronger recruitment of aged neutrophils to inflamed organs even if observed chemokine receptors are not upregulated on the cells. Interestingly, among other molecules involved in adhesion, CD44 levels were also increased on aged neutrophils. This molecule is required for neutrophil accumulation in the systemically inflamed liver and thus might rationalize the absolute dominance of aged vs nonaged neutrophils in this organ during LPS challenge.[7]

Probably the most important question resulting from the study by Uhl et al is whether the early, and dominant, infiltration of inflamed tissues and organs by aged neutrophils is a good thing. In the study, the aged cells were shown to exhibit increased capacity to phagocytize both gram-negative and gram-positive bacteria when stimulated with LPS; however, neither respiratory burst (measured also in vivo) nor cytokine production was observed to increase in these aged cells. Considering that the excessive generation of ROS and cytokine storm are deleterious in systemic inflammation, this functional phenotype suggests that aged neutrophils play a positive role in inflammation. In contrast, Zhang et al reported that in response to LPS in vitro, aged neutrophils produce more neutrophil extracellular traps (NETs) and ROS. Considering that NETs themselves can be harmful to bystander cells,[8] strong accumulation and activity of nonaged neutrophils at the sites of inflammation might also have side effects. Importantly, in line with these latter observations, in some conditions, preventing the recruitment of aged neutrophils has been shown to be protective against tissue damage.[4]

It remains to be resolved what consequences result from the recruitment of aged neutrophils prior to the recruitment of the more classical nonaged cells and the dominancy of this aged population at the inflammatory focus. This is especially important given this behavior/phenotype of aged neutrophils was observed not only following LPS challenge but also following both lipoteichoic acid challenge, a key pathogen-associated molecule pattern of gram-positive bacteria, and in response to HMGB-1, a representative damage-associated molecular pattern.[1] Therefore, this early and robust response by aged neutrophils appears to be a universal phenomenon. The upregulated expression of receptors required for pathogen recognition and recruitment certainly naturally predispose these cells to this process, but is it simply the consequence of the aged

phenotype or is it designed that these "experienced" neutrophils are called upon as the very first line of defense? These questions remain open and their answers will be key to our understanding of inflammation, tissue damage, and repair.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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DOI 10.1182/blood-2016-09-739680

● ● ● TRANSPLANTATION

Comment on Holtan et al, page 2350

Late-onset acute GVHD: clues for endothelial GVHD

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Late acute (LA) graft-versus-host disease (GVHD) has recently been added to the clinical spectrum of GVHD. In this issue of *Blood*, Holtan et al (and the Chronic GVHD Consortium) studied LA GVHD in a large prospective cohort and describe its link with circulating angiogenic factors.[1]

Thanks to the 2005 National Institutes of Health Consensus Conference diagnostic criteria of GVHD,[2] it is now widely accepted that acute GVHD can occur well beyond the classical landmark of day 100. LA GVHD can be considered as de novo if signs develop after day 100 or recurrent if signs occur beyond day 100 in a patient with a previous history of acute GVHD.

In the study by Holtan and coworkers, the authors took advantage of the Chronic GVHD Consortium Network to study, for the first time prospectively, the incidence, response to therapy, and outcome of patient with LA GVHD. Among 909 patients, 83 developed LA GVHD (2-year cumulative incidence, 11%) at a relatively early time posttransplant (around day 100). As expected, organ involvement was mostly represented by gut and skin. Systemic steroids were newly started or increased in nearly two-thirds of the case, more than half of the patients needed second-line treatment (mainly for progressive disease), and only one-fourth discontinued immunosuppressive therapy. The median failure-free survival was only 7 months and the 2-year overall survival was 56%. Finally, in analyzing the overall cohort of 909 patients, authors found that LA GVHD was associated with a 1.7-fold increased risk of overall mortality and nonrelapse mortality (NRM). All of these prospectively collected data are of significant clinical interest and will be useful for patient counseling and treatment decisions.

The strength of this study comes from ancillary studies which analyzed circulating angiogenic factors. The authors analyzed prospectively collected samples from cases (n = 55) and controls (n = 50) and data from an independent validation cohort (n = 37). They analyzed 4 epidermal growth factor (EGF) receptor (EGFR) ligands, 2 EGFR ligand sheddases, and 3 regulators of angiogenesis (see their article for details). The main result of this biological analysis was that plasma amphiregulin (AREG), an EGFR ligand, and elevated AREG-to-EGF ratio were associated with increased NRM and decreased probability of survival.

The relationship between acute GVHD and endothelium has long been discussed, with evidence of increased circulating levels of molecules like thrombomodulin, plasminogen activator inhibitor 1, pathological evidence of endothelial lesion[3] and donor-derived endothelial cells[4] in severe intestinal GVHD, or, more recently, experimental evidence of neovascularization.[5] The link between endothelial activation and the allogeneic reaction has been reviewed relatively recently.[6] Here, the investigators focused on angiogenic factors they previously studied in classical acute GVHD (before day 100).[7] Among 9 tested molecules, only AREG and an elevated AREG-to-EGF ratio correlated with clinical outcomes. It is of note, however, that these biological alterations were found not only in patients with LA GVHD but also in patients with classical acute GVHD; they were absent in patients with chronic GVHD.

The interaction between the EGFR with its ligands turns out to be extremely complex nowadays because at least 7 ligands have been described. Among them, EGF is a high-affinity ligand whereas others like AREG act as a low-affinity ligand[8] (of note only 4 have been studied by Holtan et al).

In our opinion, the most intriguing (and interesting) aspects of the study by Holtan et al are those on AREG. Emerging data suggest that AREG might be a critical component of type 2–mediated resistance and tolerance. Notably, numerous studies demonstrated that in addition to the established role of epithelial- and mesenchymal-derived AREG, multiple cell hematopoietic-derived subsets can express AREG, including mast cells, basophils, and innate lymphoid cells type 2 (reviewed in Zaiss et al[9]). Last but not least, a fascinating article by Rudensky's group recently described that, in addition to their suppressor function, regulatory T cells (Tregs) have a major, direct, and nonredundant role in tissue repair and maintenance.[10]