epidermis in CD11c-p14$_{del}$ mice in response inflammation elicited by topical 2,4,6-trinitro-1-chlorobenzene, but did not acquire LC markers. It appears that LC precursors of monocytic origin can enter the epidermis, but cannot persist to properly differentiate into bona fide LCs in the absence of p14, stopping differentiation at the pre-LC state. Because LC precursors do not proliferate and undergo apoptosis in the absence of p14, long-term LCs also cannot reside in the epidermis to form a proper network. Therefore, p14 deficiency affects all LC lineages.

The hair follicles have been demonstrated to be responsible for recruiting pre-LCs via chemokine production and to act as gateways to allow pre-LCs to enter the epidermis.[5] The authors very nicely capture this process by immunofluorescence microscopy, where short-term LCs were found to accumulate to hair follicles as they entered the epidermis.

Finally, via biochemical analyses using bone marrow–derived DCs, the authors show that mammalian target of rapamycin complex 1 and MAPK kinase 1/extracellular signaling-regulated kinase 1 signaling are impaired in the absence of p14, establishing the importance of these signaling pathways in subsets of DCs. It is interesting to think about the significance of these mechanisms in disease contexts. In vivo functions of LCs reported thus far appear to be context dependent,[8,9] and the broad picture is yet to be established. However, one could easily think of 2 clinically relevant situations: LC histiocytosis and graft-versus-host disease (GVHD). In the former, although the tumor cells are not identical to normal LCs, they do exhibit DC phenotypes,[10] and it might be worth studying whether p14 is required for proliferation of neoplastic cells in LC histiocytosis or non-LC histiocytosis. In bone marrow transplantation settings, LCs have been implicated in activating donor-derived lymphocytes to initiate acute GVHD. The current treatment of GVHD is to put patients on strong immunosuppressive agents. If, in fact, LCs have roles in initiating GVHD in humans, it is attractive to hypothesize that prior elimination of LCs by targeting signaling pathways described by Sparber et al might ameliorate GVHD or reduce its incidence in patients who have undergone bone marrow transplantation. Targeting p14 or its downstream signaling pathways might provide alternative pathways as therapeutic targets in these 2 important diseases.

DCs are central regulators of immunity. Vaccination targeting DCs is a growing and important field. However, DCs have not received as much attention as therapeutic targets in repressing inflammatory diseases. The work by Sparber et al not only represents an important finding in DC biology, but also provides impetus to designing new strategies on DC targeting, and into which diseases this concept should be applied.

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Comment on Sapey et al, page 239

A straight neutrophil path to healthy aging?

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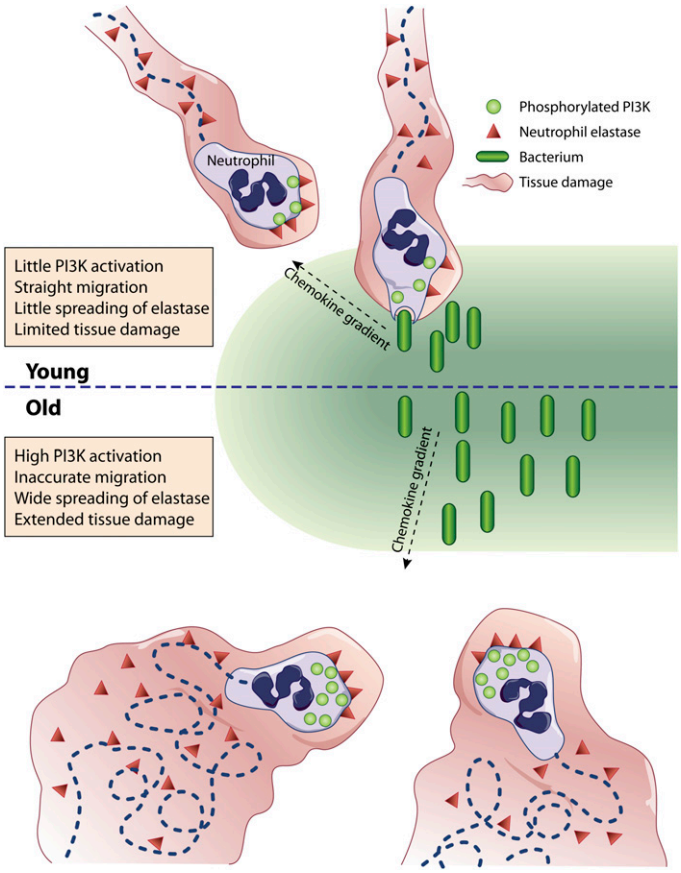
In this issue of *Blood*, Sapey et al[1] report that the human polymorphonuclear neutrophil leukocyte (or neutrophil) undergoes an age-related loss of its ability to migrate up chemotactic gradients, a functional defect that seems causally related to alterations in the polyphosphoinositide pathway.[2]

The decline in the efficiency of the immune system is a well-established consequence of aging, a phenomenon termed immunosenescence. The impact of aging on immunity has been principally addressed within the context of the acquired arm of the immune system,[3] and little is known about the senescence of neutrophils. This is surprising considering that the neutrophil occupies a central role in the initiation and regulation of the inflammatory response and in the first line of defense of the organism against injurious stimuli. The neutrophil also regulates the effector arm of the immune system by virtue of its ability to synthesize and secrete a wide variety of cytokines and chemokines. In addition, its phenotypic plasticity allows it to express functional MHC class II molecules and possibly functional T-cell receptors. These functions of the neutrophil are not fully understood or appreciated.[4] It is nevertheless clear that profound functional links exist between the neutrophil and the other actors of the immune response. Characterizing how aging impacts neutrophil functions is therefore critical if we hope to improve the immune response of the aged.

Neutrophils need to move to the site of tissue infection or injury to accomplish their



(Top) Neutrophils from young (<35 year old) individuals migrate directly up a chemotactic gradient toward an infectious focus where they phagocytose bacteria causing limited tissue injury from little release of elastase. (Bottom) Neutrophils from aged (>65 year old) individuals migrate haphazardly delaying their arrival to the infection site and causing extended tissue damage from the spreading of elastase.

functions. They are accordingly one of the most, if not the most, motile of blood cells. The ability of neutrophils to move up a chemotactic gradient in an amoeboid fashion, termed chemotaxis, allows them to be effective. In the early 1980s, Corberand et al reported the reduced chemotactic ability of neutrophils from older individuals.[5] Now, >30 years later, the molecular mechanisms involved in this dysfunction begin to unravel.

In a methodical series of experiments, Sapey et al provide evidence that blood neutrophils isolated from a large sample of donors of different ages (21-89 years) gradually lose, not their intrinsic motility, but their ability to move accurately up a concentration gradient of the major defined chemotactic factors, as well as toward sputum collected from patients with confirmed *Streptococcus* pneumonia infection. A constitutive activation of phosphatidylinositol 3-kinase (PI3K) in neutrophils isolated from older (>65 year old) donors (as evidenced by the tyrosine phosphorylation of the p85 regulatory subunit of class IA PI3K) is described. The authors then show that, although inhibition of PI3K decreases the migratory ability of neutrophils from younger donors (<35 years old), it restores the young phenotypes to the neutrophils isolated from older donors. Conversely, inhibition of the 5-polyphosphoinositide phosphatase phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1 responsible for turning off the PI3K pathway transforms young neutrophils into old ones and amplifies the old phenotype of old neutrophils. Subsequent inhibitor studies point to a specific role of a subset of PI3Ks, namely PI3Kδ and PI3Kγ, in these observations. The authors finally suggest that the inappropriate meandering of neutrophils from older donors is associated with increased membrane-associated (or shed) proteinase activity and degradation of tissues and/or extracellular matrices (see figure).

The description of the loss of the ability of neutrophils to properly orient themselves and migrate to the appropriate site is a highly significant observation in that this functional alteration is likely to impact profoundly on their ability to exercise their protective roles in the inflammatory and immune responses. An inappropriate neutrophil recruitment and activation will limit their ability to respond to infectious stimuli. It will also negatively affect their adeptness to properly orchestrate the subsequent immune response.

Based on their inability to restore the function of old cells with young plasma, the authors propose that the old neutrophils are intrinsically deficient. The short (45 minutes) incubation period in the young environment, however, might not be long enough to reverse the putative effect of chronic exposure to an old environment. Indeed, is the described higher activation level of PI3K (that led to disrupted chemotaxis) and the increased release of proteinases (resulting in greater tissue damage) the cause or the consequence of the augmented inflammatory status in the elderly? This is intriguing, and further work directly addressing this important question is needed to determine the intrinsic and extrinsic contributions to the functional defects of neutrophils.

The data described raise a number of fascinating questions for future investigations. What are the signaling elements upstream of the tyrosine phosphorylation of p85 that are affected by aging? What are the biochemical and downstream signaling consequences of the increases in phosphatidylinositol(1,4,5)-trisphosphate [PtdIns(1,4,5)P_3; the product of the activity of PI3Ks] that are suggested by the data presented (this is a major limitation of the present studies as it has yet to be established)? What are the temporal and spatial distributions of PtdIns(1,4,5)P_3 in resting and stimulated neutrophils [PtdIns (1,4,5)P_3 transiently accumulates, among others, at the front of moving cells and at the base of the phagocytic cups during particle internalization (phagocytosis)]? What are the respective contributions of PI3Kδ and PI3Kγ? Why and how do the neutrophils lose their "compass" on hyperphosphorylation of p85? Is there a causative link between the dysregulation of the PI3K pathway and the increased proteinase activity? Does the altered regulation of the PI3K pathway lead to other phenotypic changes in neutrophils such as

altered life span or the potential of acquiring the ability to interact with the other components of the immune system? Do similar events occur in other immune cells on aging and with what consequences? An affirmative answer to the latter question would indicate that treatments targeting the PtdIns(1,4,5)P_3 pathway would have consequences on the immune system broader than just the restoration of migration accuracy of neutrophils.

By identifying specific PI3Ks involved in the ability of neutrophils to migrate appropriately, these studies go beyond a descriptive analysis of a phenomenon of basic, as well as clinical and societal, relevance. The reversal of the old phenotype by PI3K inhibitors suggests avenues of research for improving disease outcomes in aging patients.

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● ● ● TRANSPLANTATION

Comment on Schuetz et al, page 281

Tearing RAGs apart

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In this issue of *Blood*, Schuetz et al analyze the immunologic and nonimmunologic outcomes in cohorts of severe combined immunodeficiency (SCID) patients with either RAG or ARTEMIS mutations following allogeneic hematopoietic stem cell transplantation (HSCT).[1] The authors show that full immunologic recovery is more likely to be achieved if myeloablative conditioning is used; additionally, they show that in ARTEMIS patients, the use of alkylating chemotherapy agents is associated with a higher incidence of nonimmunologic complications. The challenge remains, especially in ARTEMIS deficiency, to achieve full immunologic recovery without long-term complications. The use of novel conditioning agents or the development of gene therapy strategies may help improve overall outcome for these patients.

Very few articles have the ability to change clinical practice. This article by Schuetz et al makes a strong argument to be one on not just 1, but 2, clinical issues.

SCIDs are a genetically heterogeneous group of conditions characterized by the absence of T cells, varying degrees of B-cell development (often categorized as T-B+ or T-B- forms), and presentation with severe recurrent infections in the first year of life. Definitive treatment by HSCT has shown increasing success over the years[2] and the absence of adaptive T-cell immunity in SCID has allowed HSCT to be undertaken without any chemotherapy conditioning, especially if a matched sibling donor is available.

Until very recently, the data on SCID HSCT outcome have been pooled together for all forms of SCID. However, treating physicians have long recognized that some SCID forms do better than others and also that long-term outcomes are different. Furthermore, in this genomic era and with the recognition of 20 different SCID gene defects[3] (at the last count), there is a need to tailor treatments and analyze outcomes according to the genetic form of SCID. The collaborative efforts of 3 centers in Ulm, Paris, and San Francisco finally allow us to make more rational treatment decisions and understand outcomes more clearly for the RAG and ARTEMIS forms of SCID.[1]

In both RAG and ARTEMIS SCID, the underlying molecular defect is associated with DNA breakage and repair. Importantly, RAG genes are lymphoid specific and are involved in the process of V(D)J recombination whereas ARTEMIS is ubiquitously expressed and is part of the nonhomologous end-joining (NHEJ) repair mechanism of all cells.[4] Nevertheless, both forms are immunologically characterized by the absence of T and B cells (T-B- SCID) and so have been treated similarly.

In this study, the overall survival outcomes are equivalent between the RAG and ARTEMIS types. However, the first major issue that this article highlights is the poor immunologic recovery in both these SCID forms following unconditioned HSCT. Without conditioning in the matched sibling/relative donor setting, only 50% of patients achieved T cells of >1000 cells per μL, only 42% had CD4 T cells >600 cells per μL, and 44% still receive immunoglobulin replacement. It is also likely, though not measured in this study, that there was minimal naive T-cell recovery. In both SCID forms, the molecular defect results in impairment of V(D)J recombination in thymocyte and B-cell progenitors. Thus, although thymocyte development is very abnormal, the thymic niche remains occupied and, similarly, B-cell progenitors occupy the bone marrow (BM) but do not develop. HSCT without chemotherapy conditioning is therefore unable to allow effective engraftment of donor progenitor cells in the thymus and even less so in the BM. As might be predicted, immune recovery is limited to mature T cells, which have a limited repertoire and provide limited B-cell help. In the haploidentical setting for both RAG and ARTEMIS SCID, HSCT with no or limited conditioning has even worse outcomes with very poor levels of engraftment and T-cell recovery, no B-cell recovery, a high rate of second HSCT, and poor overall survival.

Thus, clinical practice message 1 is that, in RAG and ARTEMIS SCID, myeloablative conditioning is required to achieve long-term T- and B-cell recovery. Unconditioned HSCT allows only limited immune recovery in the matched sibling/related donor