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.

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● ● ● 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]

Thus, as is usual in good science, the study by Holtan and coworkers raises more questions than answers: what are the sources of AREG (epithelial, endothelia, Treg, …)? Is that a general phenomenon in acute GVHD that the host uses for tissue repair? And, last but not least, are endothelial cells truly targeted by direct or indirect allogeneic recognition or simply damaged by inflammatory mediators?

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VASCULAR BIOLOGY

Comment on Rossi et al, page 2359

"Fishing" out the real VEGFs

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In this issue of *Blood*, using an elegant zebrafish-based model, Rossi et al demonstrated that vascular endothelial growth factor B (VEGFB), VEGFD, and placenta growth factor (PlGF) are able to sustain vascularization in the absence of VEGFA and generated dominant-negative VEGF mutants, thereby identifying new antiangiogenic strategies. There are multiple important points in this article which not only change our current understanding of the mechanisms of vascular growth in a complexity of vivo settings, but also advance the search for promising therapeutic approaches seeking to interfere with pathological vascularization.[1]

Discovery of VEGFA as a key permeability and angiogenic factor produced by tumors and driving excessive pathological vasculature created a new front in the battle against cancer and other diseases associated with hypervascularization.[2,3] The prominent role of VEGFA in developmental vascular growth is underscored by severe defects leading to embryonic lethality caused by the loss of a single allele of VEGFA.[4] Successful targeting of VEGFA with neutralizing antibodies is generally viewed as revolutionary and a turning point in antiangiogenic therapy.[3] There are, however, situations when cancer develops resistance to VEGF inhibition, often by utilizing alternative proangiogenic pathways.[5] VEGFA and other VEGF family members act through main receptors on the endothelial cells of blood vessels: VEGF receptor 1 (VEGFR1), VEGFR2, and Neuropilin 1 (NRP1) (see figure). Despite decades of research, the functional redundancy and exact in vivo roles of individual VEGF family members and their receptors is either unclear or controversial.[6,7]

Accordingly, Rossi et al aimed to assess whether and how various members of the VEGF family are able to support vascular development when VEGFA activity is disrupted. To this end, using a contemporary state-of-the-art genetic approach, Rossi et al created an efficient model by mutating *vegfaa* and *vegfab* in zebrafish where vasculature is visualized by enhanced green fluorescent protein (EGFP) expression. Thorough characterization of these mutants revealed that inactivation of *vegfaa* resulted in a number of severe vascular defects eventually causing lethality. This recapitulated the consequences of VEGFA loss in mammals,[4] whereas the phenotype of the *vegfab* mutant was substantially milder. Injection of messenger RNA (mRNA) encoding Vegfaa-121 and -165 rescued the gross defects in arteriogenesis but not the formation of intersegmental vessels. This allowed the authors to conclude that Vegfaa might be dispensable in adult zebrafish, and then use the resulting mutants as a powerful tool for assessing the angiogenic redundancy of VEGF family members in physiologically appropriate settings. Thus, Rossi et al created and validated a new in vivo system for even wider screening of potentially proangiogenic molecules that are able to bypass the requirement for VEGFA. It is remarkable that in this system, *vegfd*, but not *vegfc*, was able to rescue vascular defects via VEGFR2.

Another important finding is that growth factors Pgfb (analog of mammalian PlGF) and Vegfbb (analog of mammalian VEGFB), which are dispensable for vascular development,[8] were able to compensate for missing Vegfaa. Therefore, targeting the PlGF/VEGFR1 pathway during VEGFA blockade seems to be well supported by in vivo genetic evidence, thereby addressing yet another controversial topic.[8,9] Furthermore, using a series of Vegfaa mutants generated based on the crystal structure of VEGF-VEGFR2 and VEGF-NRP1 complexes, Rossi et al established the key role for Vegfaa-VEGFR2 interaction rather than the Vegfaa-Nrp1 axis in vasculogenesis. These experiments used a combination of genetic approaches in vivo with a structure-function analysis of growth factor interactions with their respective receptors, and thereby provided a mechanism to answer important questions in the field of vascular biology, including but not limited to the relative contributions of VEGFR2 vs Nrp1 and the role of Vegfaa-165 vs Vegfaa-121 in vascular development. Notably, similar experiments in mammals are either extremely time-consuming or practically impossible. Because these