Then, the authors elegantly performed a side-by-side comparison of the antigen presentation capacity among mature neutrophils and autologous DC and monocyte subpopulations, isolated from either the blood of human donors or the LNIs and spleen of immunized rhesus macaques. As expected, neutrophils resulted in the least efficient, but still valid, APCs. In vivo, however, neutrophils, as well as monocytes, corresponded to the most numerous Env+ cell type readily detectable at the muscle injection site and in the dLNIs of the arm of immunized rhesus macaques, suggesting that, with their high number, they might overcome their poor antigen-presenting capacity compared with professional APCs.

All in all, the study by Vono et al adds convincing experimental evidence that reinforce the view of neutrophils as cells also involved in adaptive immune responses. Nonetheless, further studies are necessary to definitively clarify how important neutrophils are in the real life in functioning as APCs. Moreover, the precise molecular mechanisms whereby the cognate antigen and antigen-specific memory CD4+ T cells promote the expression of MHC-II and costimulatory molecules in mature neutrophils need to be elucidated. Obvious candidates are the T-cell–derived cytokines (see figure). Similarly, it is not completely clear how mature neutrophils could activate antigen-specific memory CD4+ T cells. Vono et al show that anti-HLA-DR antibodies prevent T-cell proliferation. However, besides the costimulatory molecule-mediated signals, other T-cell–activating factors might be soluble mediators/cytokines derived from cognate antigen/T-cell–activated neutrophils (see figure). Another open question is whether all neutrophils or only a specific subpopulation bear antigen-presenting capacity. Vono et al’s study appears to be in favor of the former hypothesis, as the authors show that the few mature neutrophils isolated from the low–density fraction of leukocytes (low-density neutrophils), after blood centrifugation of the same donors, functioned as APCs as efficiently as the normal-density mature neutrophils. By contrast the existence of specialized neutrophil subpopulations, exhibiting the hybrid phenotypic and functional characteristics of both neutrophils and DCs, including antigen presentation capacity, however, originating by immature neutrophil precursors, were reported. Altogether, the latter and other studies therefore indicate that different conditions, environments, and mechanisms may drive the generation of a variety of antigen-presenting neutrophil subpopulations. Finally, could all of these findings translate into a clinical application utilizing newly generated, specific neutrophil subpopulations in boosting T-cell responses in infectious disease or tumor patients? Again, future research in the field will give us the answer. Clearly, our knowledge on the biology of neutrophils will never end.

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**REFERENCES**


Thus, platelet CLEC-2 plays various roles beyond clotting.\(^2\)

Although platelet CLEC-2 is highly and specifically expressed in platelets/megakaryocytes, its role in normal hemostasis seems to be minimal, and its role in arterial thrombosis has been controversial. To date, 3 studies have reported that CLEC-2 deficiency in mice does not cause a significant increase in tail bleeding.\(^3\)\(^-\)\(^5\) CLEC-2 deficiency causes significant inhibition of arterial thrombosis in some experimental settings,\(^3\)\(^,\)\(^6\) but not in other settings.\(^4\)\(^,\)\(^6\) Lack of podoplanin expression in the normal vascular wall may explain this phenomena, although it is expressed in the atheromatous plaque in humans and mice.\(^5\)

Recently, the role of platelet CLEC-2 in maintaining vascular integrity during inflammation has been reported. Boulaftali et al reported that platelets lacking signals from G-protein–coupled receptors, but not CLEC-2–deficient platelets, rescued inflammation-induced hemorrhage in thrombocytopenic mice.\(^7\) They hypothesized that platelets leak from hyperpermeable vessels during inflammation and that CLEC-2 signaling is activated by podoplanin, whose expression is induced in the tissue around these vessels or infiltrating macrophages by inflammatory cytokines. Platelet activation by CLEC-2/podoplanin interaction may inhibit hemorrhage through unidentified mechanisms. Herzog et al demonstrated that platelet CLEC-2 also maintains the integrity of high endothelial venules (HEVs) in lymph nodes with the finding that CLEC-2-deficient mice show blood-filled lymph nodes.\(^8\) Circulating lymphocytes continuously enter into the lymph nodes from HEVs for immune surveillance, especially during the immune response. Fibroblastic reticular cells around HEVs stimulate platelets by binding to CLEC-2 via podoplanin. Sphingosine-1-phosphate released from activated platelets promotes vascular endothelial cadherin expression on HEVs, which is essential for HEV integrity.

These studies show that inflammation results in leaky blood vessels and may induce podoplanin expression at the site of the inflammations. Payne et al described deep vein thrombosis (DVT) as hypoxia-induced sterile inflammation. Hypoxia develops as a result of flow distortion and stimulates von Willebrand factor release from Weibel-Palade bodies in vascular endothelial cells, which leads to the adhesion of platelets and neutrophils with resultant thrombosis and inflammation. Payne et al found that podoplanin expression is upregulated in the venous wall during this hypoxia-induced sterile inflammation. They demonstrated that the interaction between the upregulated podoplanin and platelet CLEC-2 stimulates venous thrombosis using a DVT model of partial inferior vena cava stenosis with CLEC-2–deficient mice and anti–podoplanin blocking antibody (see figure).

A similar role of CLEC-2 in “unsterile” inflammation has been demonstrated in Salmonella infection–induced thrombosis. In bacterial infection, podoplanin is also upregulated in monocytes accumulating in the liver, which causes liver thrombosis mediated through CLEC-2.\(^5\) Shirai et al recently reported extensive thrombus formation in lung in cancer-bearing mice, which was greatly reduced in CLEC-2–depleted mice, which also showed decreased plasma levels of inflammatory cytokines.\(^10\) It is tempting to speculate that podoplanin was upregulated in the venous wall during cancer-mediated inflammation.

Thus, CLEC-2 may play a major role in thromboinflammation but a relatively minor role in arterial thrombosis and only a minimal role in normal hemostasis. Thromboinflammation takes a relatively long time to develop, whereas arterial thrombosis and normal hemostasis develop within minutes. Long-term exposure of any inflammatory cytokines may induce podoplanin expression at the site of inflammation.

CLEC-2 may be a good target for the treatment or prevention of thromboinflammation, because CLEC-2 deficiency does not cause significant bleeding tendency. However, a number of issues remain to be solved. For example, what types of cells in the vessel wall upregulate podoplanin expression? What kinds of stimuli under inflammatory conditions upregulate podoplanin expression in the vessel wall? How does platelet CLEC-2 interact with podoplanin expressed inside the vessel wall? Are there any stimulators of platelet CLEC-2 other than podoplanin? Further studies are required to resolve these issues.
In Greek philosophy, the term kairos (καιρός) defines the perfect time point. With regard to therapeutic strategies and decisions in medicine, the determination of the kairos is equally important as the identification of major mechanisms and pathways leading to substantiated understanding of (patho)physiological processes and to identification of potential therapeutic targets. In GVHD, development of novel treatment strategies is urgently needed because it remains the leading diagnostic and therapeutic challenge following allogeneic hematopoietic stem cell transplantation with the consequence of severe morbidity and nonrelapse mortality, especially when nonresponsive to high-dose steroid therapy. In recent years, evidence has emerged that the vascular endothelium could be a target structure for GVHD therapy because an association of the formation of new blood vessels and GVHD has been observed. Per se, the important role of neovascularization has long been established in GVHD, but the chronological sequence of the establishment of new vasculature and immune cell infiltration has remained unclear. Although it has been previously believed that angiogenesis would be stimulated by secreted mediators from tissue-infiltrating immune cells, Riesner and coworkers observed that the contrary is the case, showing that angiogenesis precedes the infiltration of immune cells into damaged tissues. Importantly, after incipient tissue damage, angiogenesis occurred as early as 2 days after allogeneic hematopoietic stem cell transplantation, indicating that the pathogenesis of GVHD is already commenced early with novel vasculature to tackle a route for immune cells. Their findings suggest a new chronological sequence of GVHD initiation (tissue damage, neovascularization, immune cell infiltration). These observations might help to improve our understanding of the underlying mechanisms of GVHD. Hence, this new evidence might help explain why GVHD primarily affects tissues that are more susceptible to infectious agents, why GVHD mainly manifests in distinct organs, and how GVHD-mediated T cells are primed and activated, shedding new light on immunobiology in general and GVHD T-cell biology in particular (see figure).

GVHD is believed to be mainly mediated by allogeneic T cells that recognize differences in tissue antigens, which are expressed on recipient cells. These are most importantly major histocompatibility complexes (MHCs, in humans termed HLA) as a complex with their respective bound peptides. This direct recognition of peptide MHC is avoided by MHC-matched (or HLA-identical) transplantation. However, about 40% of patients who receive HLA-identical grafts will still develop GVHD, which can then be mediated by recognition of minor histocompatibility antigens. These constitute MHC-presented peptides representing protein fragments which are produced during normal cellular metabolism and exhibit amino acid sequence variants based on single-nucleotide polymorphisms between donor and recipient. Further recognition of peptides can also be based on specific sequence variants based on intrinsic alterations in leukemic cells, then mediating graft-versus-leukemia (GVL) effects. Of note, sole recognition of such antigen differences cannot be the only mechanism mediating GVHD or GVL because it has been observed that new autoimmunity (diseases sharing features with “naturally occurring” autoimmune diseases) and graft versus self (GVS; recognition of MHC-restricted autoepitopes) can develop, constituting that further processes must be involved in the genesis of graft-host interaction.

Based on the findings by Riesner et al, neovascularization could hereby be an important prerequisite for priming of naive T cells or for proliferation of tissue-specific memory T cells against alloantigens, autoantigens, and leukemia (or tumor) antigens. This is further underlined by the observation that endothelial cells can also upregulate MHC molecules and function as antigen-presenting cells to naive T cells in inflamed tissue. The data of Riesner and colleagues might in this context also support a further comprehension of in situ T-cell priming. Importantly, they used a...
CLEC-2/podoplanin and thromboinflammation

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