Natural Killer Cells in Pulmonary Arterial Hypertension
A Force on the Dim or the Bright Side?

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Pulmonary arterial hypertension (PAH) is a progressive disorder of the pulmonary vasculature characterized by the constriction of precapillary pulmonary arteries, leading to sustained elevated pressure in pulmonary vessels. The chronic strain and then failure of the right heart leads to significant morbidity and mortality in affected patients. In untreated patients the prognosis can be as poor as in patients with malignancies.

The pathophysiology of PAH is unclear. Histological features reveal proliferation of endothelial and smooth muscle cells with vascular remodeling resulting from the formation of plaquelike lesions with fibroblasts, smooth muscle cells, and endothelial cells. Dysregulated angiogenesis, morphological and functional alterations of microvessels are hallmark features of chronic inflammatory disorders. It is generally accepted that peripheral and coronary vascular disease is linked strongly to inflammatory processes.1 There is growing evidence for a fundamental involvement of inflammatory processes in PAH. For decades it has been recognized that immune phenomena are associated with PAH.2 Inflammatory cells and intense chemokine production have been detected within remodeled pulmonary arteries, but it has not been clear whether the complex phenomenon we call inflammation surrounding the pulmonary vasculature is primarily the cause or the consequence of vascular remodeling.

Perivascular T and B lymphocytes have been detected in pulmonary vascular PAH lesions.3 A recent study further shows that pathogenic antibodies and corresponding T cells may also be generated locally in the lung, in highly organized ectopic lymphoid follicles, with stimulating autoantibodies as possible mediators of PAH,4 which suggests that the adaptive immune system is involved. Although a role of the adaptive immune system, especially of T lymphocytes, in the initiation and progression of PAH was demonstrated, the contribution of effectors of the innate immune system has only recently come into focus.

In the current issue of Circulation, Ormiston et al5 focus on an aspect of PAH that has been neglected in the past—the role of innate immune mechanisms in PAH and, in particular, the role of natural killer (NK) cells in PAH.

NK cells are enigmatic cells situated at the frontier between innate and adaptive immunity. Because they have germ-line encoded receptors, they seem to belong to innate immunity (in contrast to cells that bear rearranged receptors); however, they might have memory, a typical function of adaptive immunity. So the line between innate and adaptive is a bit blurred with regard to NK cells. In humans, NK cells are usually identified phenotypically as CD56+/FcγRIII (CD16+)/CD3− cells and occur in 2 subsets in the blood—the abundant CD56dim (∼95%) and the rare CD56bright (∼5%). They represent 10% to 15% of circulating lymphocytes in the peripheral blood.6 NK cells play a pivotal role in innate immunity (eg, by limiting viral infections before adaptive immune processes are initiated). NK cells also seem to influence directly other innate immunity effector cells such as macrophages and dendritic cells. They are tightly regulated by activating and inhibiting surface receptors that hold their potent effector function on a leash. Natural cytotoxicity receptors almost exclusively restricted to NK cells and comprising NKp46, NKp30, and NKp44 play an important role in the killing of tumor cells. Ligands for these receptors are as yet unknown, with the exception of B7-H6, a ligand for NKp30.7 Furthermore, killer immunoglobulin-like receptors (KIR) can be found on NK cells. Each KIR has as its ligand a subgroup of human leukocyte antigen class I allotypes, and each KIR displays 2 (KIR2DL) or 3 (KIR3DL) extracellular immunoglobulin domains conferring specificity for HLA-C or HLA-A/B allotypes, respectively.8 These receptors can be activating or inhibiting. The net result of activation or inhibition is dependent on a plethora of receptor–ligand interactions on the NK cell surface. Some of these interactions are quite clear; others are less well established.

NK cells add an important feature to immunity. In the classical model of self/nonself discrimination, T and B cells, effectors of adaptive immunity, carry receptors and if these are engaged, killing of the infectious agent or allograft rejection results. The late Charles A. Janeway developed the idea that the immune system basically evolved to distinguish infectious nonself from noninfectious self.9 He predicted that (germ-line encoded) receptors sense molecular patterns that are shared by classes of infectious agents, such as bacterial lipopolysaccharide or viral RNA. This has led to the discovery of Toll-like receptors, brought specificity to innate immune mechanisms, and has changed our view of self/nonself discrimination.10 These mechanisms all rely on the presence of something that does not belong to self. NK cells have a different mechanism to recognize self, and this mechanism closes a gap in immunity. If self molecules are missing, the lack of inhibition allows dominance of activating receptors that drive NK cells to kill, phagocytose, and so on.

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The levels of class I molecules on a host’s cell are monitored by NK cells, and downregulation of these molecules (eg, by viral infection) leads to killing. This concept was first proposed by Klas Kärre et al11 in view of their findings of NK cells requiring binding to major histocompatibility complex molecules to be prevented from cytoxicity.

An interesting aspect of NK cells with regard to vascular remodeling and integrity is their role in pregnancy. CD56bright NK cells of the uterus constitute 70% of the leukocytes of the first trimester decidua.12 In contrast, B cells are absent and T cells are very low in number in the human uterus. Because pregnancy in mammals usually occurs without prior tissue matching, as for example, in solid organ transplantation, the immune system of the host (ie, the mother) needs to be much downregulated, at least locally. Without this downregulation, the allograft (ie, the fetus) would be lost in a host-versus-graft reaction. Uterine NK cells seem to play a role for the immunological tolerance of the fetus. In addition, because of the invasive hemochorial placentation in humans, transformation of the spiral arteries into low-pressure, high-blood flow vessels with concomitant, extensive decidualization of the uterine mucosa takes place. Uterine NK cells are present whenever decidualization takes place, also in extrauterine gravidity. Hence, it may be assumed that 1 task of NK cells, at least in the uterus, is to govern vascular remodeling and integrity or to do the vascular remodeling itself.13 The exact role of uterine NK cells, however, is not known. And we must not forget that they finally differ from blood NK cells. Pregnancy is mostly a devastating event in patients with PAH, with high mortality during the first month after delivery, with the main causes of death being heart failure and sudden death. So far it is unknown, if the only reason for disease manifestation or progression during pregnancy is the fact, that normal physiological changes of pregnancy can’t be tolerated, or whether there are additional factors like the development of maternal autoimmune disease.

**NK Cells in PAH**

In the current work, Ormiston et al3 found that the dim subset of circulating NK cells (CD56dim/CD16+) was decreased whereas there was no change in the bright NK cells (CD56bright/CD3−), which produce large quantities of inflammatory cytokines on stimulation. This led, similarly as in HIV patients, to a proportional increase in circulating CD56−/CD16+ NK cells when compared with healthy donors. Approximately 90% of peripheral blood and spleen NK cells are CD56dim/CD16+ and express perforin. CD56dim NK cells are cytotoxic and produce interferon-γ on interaction with tumor cells in vitro14; in contrast, most NK cells in lymph nodes and tonsils are CD56bright/CD16− and lack perforin.15 CD56bright cells produce high amounts of interferon-γ on stimulation with interleukin 12, interleukin 15, and interleukin 18.16 Uterine NK cells with their proposed role on vessels resemble these CD56bright cells more.

Analysis of activating and inhibitory receptors on isolated NK cells provided a mixed picture with a decrease of the natural cytotoxicity receptors NKp46, KIR3DL1, and KIR3DL1/S1, whereas there were no changes in DNAX accessory molecule-1, NKP30, or NKG2D on NK cells from patients with PAH compared with control subjects. Especially KIR3DL1+ NK cells and also their respective KIR3DL1 mean receptor expression were reduced in PAH; notably, in 3 patients these cells were completely lacking.

It might be worthwhile to monitor closely the patients without KIR3DL1+ cells, because extreme phenotypes sometimes might provide insight that would otherwise be lacking. The dysregulation was not the result of KIR or HLA gene polymorphisms in patients, which were checked by genotyping a cohort of white patients with PAH and ethnicity-matched control subjects. Instead, Ormiston et al proposed the involvement of transforming growth factor-β signaling for KIR surface regulation. In addition, a significant increase in the expression of matrix metalloproteinase 9 when compared with healthy donors was found. NK cells from patients with PAH also produced elevated quantities of matrix metalloproteinase 9 consistent with a capacity to influence vascular remodeling. Interestingly, Ormiston et al found increased urokinase gene expression in NK cells from PAH cells. Urokinase can be regarded as a surrogate marker for transforming growth factor-β signaling. The authors conclude that increased matrix metalloproteinase production by NK cells might be a protective mechanism of the remaining NK cells in PAH to protect, hypothetically, from increased muscularization of pulmonary arteries. Results from rodent models of PAH supported the observations made in humans.

Ormiston et al3 noted a functional impairment of NK cells with regard to macrophage inflammatory protein 1β secretion and degranulation. Surprisingly, the authors did not investigate secretion of growth factors such as vascular endothelial growth factor C, placental growth factor, angiopoietin 2, and others involved in angiogenesis that are, for example, secreted by uterine NK cells.

Ormiston et al3 propose a potentially important role for NK cells in the regulation of vascular remodeling underlying PAH. A limitation of their study might be that, in humans, only circulating NK cells but no lesional sites in pulmonary vessels and lymphoid tissues (eg, from transplant patients) were examined. Studies in patients with psoriasis, a common chronic inflammatory skin disorder that has been associated with endothelial damage, arterial hypertension, chronic obstructive pulmonary disease, and elevated pulmonary arterial pressure, have shown that the frequency of NK cells are decreased in the peripheral blood but significantly increased in lesional skin.17,18 In contrast to PAH, both dim NK cells (CD56dim/CD3−) and also bright NK cells (CD56bright/CD3−) were decreased in peripheral blood of patients with psoriasis, possibly because of the high concentrations at lesional sites. Injection of circulating NK cells isolated from psoriatic donors into nonlesional skin grafted in immunodeficient mice was sufficient to induce psoriasis-like lesions with angiogenesis and functional alterations of microvessels as hallmark features. Previously, Ormiston et al19 found that the presence of NK cells is required to prevent monocrotaline-induced endothelial damage by infusion of human endothelial progenitor cells, supporting the idea of an integral role for NK cells in the endothelial progenitor cell-mediated prevention of monocrotaline-induced PAH.19

In another recent study,23 samples from explanted lungs from patients with PAH were analyzed and compared with
circulating lymphocytes of the same patients. Patients with PAH had lower frequencies of circulating B lymphocytes and helper T lymphocytes than control subjects. Although control lungs showed scant detectable pulmonary accumulation of lymphocytes or inflammatory cells, lung sections from patients with PAH showed large accumulations of lymphocytes resembling highly organized lymphoid follicles that were distributed throughout the pulmonary vasculature, from small distal remodeled arteries to larger pulmonary arteries. Ormiston and colleagues\(^5\) nevertheless show that chronic hypoxia decreases NK cells in blood, spleen, and lung of mice.

Examination of NK in lymphoid organs, however, might also be important, because NK cells can kill immature dendritic cells, thus preventing the survival of less-immunogenic immature dendritic cells, which would induce inappropriate, low-affinity T-cell priming.\(^20\) Similarly, it has been shown that the transplantation of alloreactive NK cells can suppress T-cell-mediated graft-versus-host disease by eliminating host dendritic cells, resulting in a state of tolerance, or vice versa in the case of functionally impaired NK, to progression of chronic inflammatory diseases such as PAH. For the time being, the question about the exact pattern of NK cells in PAH diseased lung remains unanswered.

Ormiston et al\(^3\) are the first to show alterations in NK cell numbers and function in human PAH. Being the first report, it is almost natural that the work raises more questions than it answers: Is the NK cell physiologically important for pulmonary vessel function? Are CD56\(^{dim}\) cells, in fact, good for vascular integrity and is it bad to have less of them? Or are CD56\(^{dim}\) cells in contrast a dark force that is not actually reduced in number but has only disappeared from the peripheral blood to cause damage in pulmonary vessels? Another question is: Where, exactly, do these NK cells do their work? Is it the vessel, the matrix, or other immune cells in the lung - or is it nothing of all? Is the mixed picture of altered expression of activating and inhibiting receptors that we see in patients with PAH tipping the scales in 1 direction or another? And if so, what does it mean for PAH? Is the potential to secrete angiogenic factors of these NK cells also impaired functionally? Ormiston et al\(^3\) report a functional impairment of NK cells but no correlation with disease severity. This strong impact, however, might be beyond the predictive possibilities of a single cell in a complex disease such as PAH. But above all, the question remains whether the NK cell in PAH is a driver of disease or a cell that is trying to prevent more damage, or is merely a bystander that is forced to alter surface receptor expression and function by cytokines that are being produced where the heat is on. Surely, there are even more questions that will arise and much work seems to be ahead. As mentioned earlier, examining more patients with PAH with extreme NK cell phenotypes might bring more light to the role of the dim cells. The article\(^5\) describes that NK cells from patients with PAH differ from those in healthy persons and shows, in conjunction with animal models of PAH, that this is very likely more than a chance finding. Advances in PAH will come from a better understanding of the different pathological features of disease. More research in the field of NK cells in PAH will bring us closer to getting a complete picture, which will bring about better therapies that might include manipulations of NK cells in PAH.

Disclosures

None.

References


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