These novel findings raise many new experimental preclinical and translational questions. What roles do CACs and EPCs play in specific types of human CVD? Do CACs and EPCs respond to AMD3100 with similar affinities, and can modifying the dose administered preferentially mobilize one or the other? Are CACs and EPCs being mobilized from the same site or via the same mechanism as these cells respond differently to AMD3100 and G-CSF? Would AMD3100 treatment be best used in preclinical vascular injury models if used sequentially with G-CSF or used in some concomitant strategy or perhaps with another angiogenic agent? Do we have enough knowledge about the effects of AMD3100 and/or G-CSF in preclinical CVD models to pursue human clinical trials? How the use of AMD3100 enters into the mix of strategies to augment neovascularization in patients with CVD remains to be determined, but the data presented by Shepherd et al open a new investigative pathway.

The author declares no competing financial interests. ■

REFERENCES

1. Rafii S, Lyden D. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. Nat Med. 2003;9:702-712.
2. Ingram DA, Caplice NM, Yoder MC. Unresolved questions, changing definitions, and novel paradigms for defining endothelial progenitor cells. Blood. 2005;106:1525-1531.
3. Hristov M, Weber C. The therapeutic potential of progenitor cells in ischemic heart disease: past, present and future. Basic Res Cardiol. 2006;101:1-7.
4. Dimmeler S, Zeiher AM, Schneider MD. Unchain my heart: the scientific foundations of cardiac repair. J Clin Invest. 2005;115:572-583.

TRANSPLANTATION

Comment on Rüster et al, page 3938

MSCs roll into the mainstream

Darwin J. Prockop TULANE UNIVERSITY HEALTH SCIENCES CENTER

In research as in other human endeavors, there is always a reluctance to accept radically new ideas until the evidence becomes overwhelming. It is not surprising, therefore, that there was great skepticism about initial reports indicating that cells from the bone marrow and other tissues of adult organisms can serve to repair and regenerate tissues.

Henschler and colleagues have provided data to help resolve previously controversial observations about engraftment in vivo of the plastic-adherent cells from bone marrow referred to in the hematologic literature as marrow stromal cells, but first defined as fibroblastoid colony-forming units, then as mesenchymal stem cells, and most recently as multipotent mesenchymal stromal cells (MSCs).[1] Henschler et al used a parallel plate flow chamber to demonstrate that MSCs extended podia, rolled, and then formed firm adhesions to endothelial cells in a manner similar to both peripheral blood mononuclear cells and $CD34^+$ hematopoietic progenitors. Therefore, as the authors state, their results established that "MSCs fulfill essential prerequisites for tissue-specific extravasation and homing." The results move us a step further to understanding how MSCs and related cells from bone marrow have produced encouraging results both in animal models and in clinical trials for several diseases including osteogenesis imperfecta, graft-versus-host disease, and heart disease.

The process of homing, extravasation, and engraftment of MSCs into tissues has been a controversial issue for many years. There have been several reasons for the controversy. One reason is that the experiments first describing engraftment after systemic infusion were carried out in very young mice that had received lethal irradiation.[2] Subsequent experiments demonstrated that the levels of engraftment were very low in adult animals that did not have extensive tissue injury.[3]

A second reason for the controversy is that MSCs show large interspecies differences and they are particularly difficult to isolate from mice.[4] Therefore the results with MSCs from different species were frequently not comparable, and many critical tests in mice were not informative.

A third and related reason for the controversy is that the properties of MSCs change dramatically as they are expanded in culture even if prepared as single-cell–derived clones. There are still no definitive markers for the cells. As a result, the properties of the MSCs prepared in one laboratory differed from those in another, frequently without the investigators being aware of the differences. The problem was highlighted by our recent demonstration that the small, spindle-shaped MSCs present in low-density and early-passage cultures engraft more efficiently than the larger MSCs that appear as the cultures approach confluency.[3]

A fourth reason for the controversy is that it is difficult to identify markers to follow engraftment of MSCs as they begin to differentiate in tissues in response to the local microenvironments. Most exogenous labels are fickle in that dye- or iron-based markers can be lost as the cells propagate or are transferred to macrophages and other cells in vivo. Genetic labels such as green fluorescence protein can also generate artifactual results, because the gene products become toxic to cells or generate immune reactions that destroy the cells.

Finally, the controversy has been indirectly fueled by the political controversy over research on human embryonic stem (ES) cells. MSCs can be used as autologous cells and they have little risk of generating tumors that are regularly generated by ES cells. Therefore, there was concern that if MSCs or similar cells from adult tissues engrafted into tissues, the observations could be used as an argument to stop research on human ES cells.

Henschler and colleagues also demonstrated that the binding of human MSCs to human umbilical cord vein endothelial cells (HUVECs) was mediated both by P-selectin and VCAM-1. The rolling and adhesion of the cells was stimulated by TNF-α. In addition, the authors demonstrated that MSCs rolled and adhered to postcapillary venules in vivo in a mouse model in a P-selectin–dependent manner. The observations are a basis for defining the next steps in the engraftment of MSCs—that is, determining whether MSCs extravasate using the same molecular interactions used by other marrow-derived cells. As the authors point out,

the results also make it feasible to determine whether the cells can be genetically engineered so that they more effectively home to and engraft into diseased tissues.

The author declares no competing financial interests. ■

REFERENCES

1. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells: The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8:315-317.
2. Pereira RF, Halford KW, O'Hara MD, et al. Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage and lung in irradiated mice. Proc Natl Acad Sci U S A. 1995;92:4857-4861.
3. Lee RH, Hsu SC, Munoz J, et al. A subset of human rapidly-self renewing marrow cells (MSCs) preferentially engraft in mice. Blood. 2006;107:2153-2161.
4. Phinney DG, Kopen G, Righter W, Webster S, Tremain N, Prockop DJ. Donor variation in the growth properties and osteogenic potential of human marrow stromal cells. J Cell Biochem. 1999;75:424-426.

HEMOSTASIS

Comment on Pendu et al, page 3746

Sticky business: von Willebrand factor in inflammation

José A. López PUGET SOUND BLOOD CENTER

The molecular biology of inflammation takes a new turn with the report of Pendu and colleagues that von Willebrand factor binds leukocyte receptors mediating leukocyte rolling and firm adhesion.

The cardinal feature of inflammation is the migration of leukocytes from the blood into the tissue in response to chemical signals elaborated by extravascular cells or invading pathogens. These signals not only summon the leukocytes, they also prepare the endothelium to bind the leukocytes by inducing it to express adhesive molecules. Using these adhesion molecules, leukocytes decelerate by rolling; they then stop and migrate across the endothelial layer. Rolling is mediated by proteins of the selectin family that appear on the activated endothelium, either translocated immediately to the cell surface from storage granules (P-selectin) or synthesized de novo after several hours (E-selectin). The endothelial selectins bind a leukocyte counter-receptor known as P-selectin glycoprotein ligand-1 (PSGL-1). The rolling interaction slows the leukocytes and they become activated, partly through signals produced by ligation of PSGL-1. Activation changes the affinity of integrin αMβ2, which then binds endothelial counter-receptors, including ICAM-1, allowing the leukocytes to adhere firmly and exit the blood vessel. Activated platelets at sites of vessel injury also facilitate leukocyte emigration, binding both PSGL-1 and αMβ2 by virtue of high-density coatings of their respective counter-receptors, P-selectin and glycoprotein Ib.

This scenario now becomes even more complicated, with the findings of Pendu and colleagues that both PSGL-1 and αMβ2 can bind von Willebrand factor (VWF), as reported in this issue of the journal. PSGL-1 interacted weakly with VWF, accounting for transient contacts between unactivated leukocytes and VWF, whereas αMβ2 on activated leukocytes allowed them to adhere stably. Thus, one molecule, VWF, contains all of the determinants necessary to allow leukocytes to decelerate and stably adhere to a surface.

VWF is of particular interest because it is stored with P-selectin in endothelial Weibel-Palade bodies and is secreted in response to the same signals that externalize P-selectin. Earlier studies showed that VWF deficiency in mice lessened the inflammatory response in 3 diverse processes: wound healing, cytokine-induced meningitis, and atherosclerosis.[1,2] These effects were interpreted as being due largely to the mispackaging and defective secretion of P-selectin associated with VWF deficiency. The new data indicate that VWF may contribute to inflammation by directly binding leukocytes.

The importance of this inflammatory mechanism is difficult to know. For one thing, VWF is quickly removed from the endothelial surface by the metalloprotease ADAMTS13.[3] Furthermore, even if VWF persists, leukocytes have to outcompete platelets, which outnumber them by up to 60-fold. The inflammatory role of VWF could thus be largely secondary, with the adherent, activated platelets providing a surface for leukocyte recruitment. In either case, VWF could recruit leukocytes when conditions favor the persistence of its hyperadhesive forms on the endothelial surface, as occurs under the influence of inflammatory cytokines, which not only stimulate VWF secretion but also inhibit its processing by ADAMTS13.[4] It is of interest that VWF levels are elevated in both chronic and acute inflammation.[5]

These interesting findings notwithstanding, the argument for whether VWF plays an active role in inflammation or merely serves as a nonspecific marker of its existence will ultimately be settled by astute clinical observation of patients with severe von Willebrand disease, with a particular emphasis on detecting defects in wound healing or enhanced susceptibility to infection.

The author declares no competing financial interests. The author is also affiliated with the University of Washington. ■

REFERENCES

1. Denis CV, Andre P, Saffaripour S, Wagner DD. Defect in regulated secretion of P-selectin affects leukocyte recruitment in von Willebrand factor-deficient mice. Proc Natl Acad Sci U S A. 2001;98:4072-4077.
2. Methia N, Andre P, Denis CV, Economopoulos M, Wagner DD. Localized reduction of atherosclerosis in von Willebrand factor-deficient mice. Blood. 2001;98:1424-1428.
3. Dong JF, Moake JL, Nolasco L et al. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. Blood. 2002;100:4033-4039.
4. Bernardo A, Ball C, Nolasco L, Moake JF, Dong JF. Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. Blood. 2004;104:100-106.
5. Vischer UM. von Willebrand factor, endothelial dysfunction, and cardiovascular disease. J Thromb Haemost. 2006;4:1186-1193.