Peripheral Blood CD31⁺ Cells for the Treatment of Ischemic Vascular Disease*

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The past decade has witnessed an unprecedented rapidity of progression from laboratory findings to clinical trials in the field of cell therapy for ischemic disease. Trials have been completed, or are currently ongoing, in patients with acute myocardial infarction, chronic ischemic heart disease, non-ischemic cardiomyopathy, and peripheral arterial disease. Much of the original pre-clinical data that underpinned these clinical trials can be attributed to Dr. Jeffrey Isner and his group in Boston who demonstrated that peripheral blood mononuclear cells (PBMCs) were capable of assuming some features of endothelial cells after brief periods of culture (1,2). They originally referred to these cells as endothelial progenitor cells, on the assumption that the cells generated were both endothelial in nature and derived from immature circulating precursors of bone marrow origin. Whereas subsequent detailed evaluation by a variety of groups has since suggested these cells to be neither purely endothelial nor progenitor in nature (3–5), animal studies have consistently demonstrated that administration of PBMC subsets or cells cultured from PBMCs promotes angiogenesis in ischemic myocardium or limbs in addition to preserving tissue function.

There have been few head-to-head studies comparing therapeutic efficacy of different cell populations. Acknowledging this, if one interprets the available data to indicate no clear-cut advantage of 1 cell type over another, then the ease by which 1 cell type can be translated to the clinical arena becomes highly relevant. Thus, bone marrow harvesting, cytokine administration, cell culturing, genetic manipulation, and such must be regarded as barriers to clinical translation and the case for such manipulations would need to be strong if easier and quicker cell preparation strategies were equally efficacious. Recently, delivery of granulocyte colony stimulating factor mobilized peripheral CD34⁺ cells has been demonstrated to have important clinical effects in studies of intramyocardial delivery in patients with intractable angina (6).

A PBMC cell fraction not requiring in vitro culture modification or mobilization, but enriched for therapeutic efficacy, would clearly have clinical appeal. In this issue of the Journal, Kim et al. (7) report that administration of human peripheral blood CD31⁺ cells (without culture), when compared with administration of the remaining CD31⁻ fraction or of saline, promote angiogenesis and blood perfusion in a murine model of limb ischemia as well as preventing limb loss. It is important to note that the investigators did not include an unselected PBMC group in this study, so it is not possible to state whether the extra step of selecting a CD31⁺ fraction would be necessary for therapeutic effect. Indeed, even administration of CD31⁻ cells appeared to improve the perfusion ratio of ischemic limbs when compared with administration of saline alone, although with less effect than CD31⁺ cells. From both a mechanistic and translational perspective, it would be of interest to know whether avoiding a cell selection process would mitigate the therapeutic potency of noncultured cell preparations such as reported by Kim et al. (7).

CD31, also known as platelet endothelial cell adhesion molecule, is a member of the immunoglobulin superfamily (8). The rationale for the choice of CD31 as the discriminatory marker is not entirely clear, but likewise, the choice of CD31⁺ cells for evaluation in a cell therapy study is not new. Indeed, members of the same group reported in 2003, the therapeutic efficacy of cultured autologous CD31⁺ cells in a porcine model of ischemic myocardial injury (9). At that time, the stated reason for choosing CD31 as a selection marker was the lack of availability of a porcine-specific antibody to CD34, a purported marker of progenitor phenotype, rationalizing that CD31 would identify a similar population of “progenitor cells.” However, the current study shows CD34 to be expressed on only a small fraction of the isolated CD31⁺ cells. Further justification for the use of CD31 as a selection marker is the demonstration that culture of these cells in endothelial growth media yields a population of cells with similar phenotypic characteristics to the original “EPCs” as defined by Kalka et al. (2). Regardless, the choice of CD31 as a selection marker has been vindicated by the demonstration of therapeutic efficacy in both the earlier autologous porcine model and the current murine model of human cell therapy.

Kim et al. (7) also sought to track the fate of delivered human CD31⁺ cells and used fluorescent labeling of CD31⁺ cell membranes before administration and in situ hybridization for the Y chromosome in subsequent tissue analysis. Additionally, they stained tissues for endothelial markers and utilized 3-dimensional confocal microscopy.
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techniques to determine spatial localization of delivered cells in relation to existing tissue architecture. Evaluation of the data suggests that a proportion of administered CD31+ cells exhibited an intimate spatial association with neovessels, which is consistent with a supportive role in neovascularization. However, their additional claim that human CD31+ cells differentiate to form functional endothelium is not supported by the data presented. At the very least, they would need to exclude intercellular dye transfer, cells in transit, and cell fusion as alternative explanations.

So what is the nature of the CD31+ cells administered? The fluorescent-activated cell sorter profile indicates a double peak, suggesting there may even be 2 populations of CD31+ cells. Regardless, a monocytic origin seems likely for the majority. A number of surface markers including CD31, CD105, and CD141 are shared between endothelial and monocyte lineage cells. Moreover, when cultured in endothelial growth media, their subsequent phenotype is consistent with cultured monocyte lineage cells (3,4) including staining for lectin and uptake of acetylated low-density lipoprotein, features of both monocytic lineage, and endothelial cells. Although there is no convincing evidence for an endothelial progenitor phenotype in vitro and in vivo, perhaps their monocytic phenotype explains their therapeutic efficacy. Monocytes play a critical role in neo-arteriogenesis (10), may drill early tubes in ischemic tissue (11), and are capable of considerable growth factor production. Indeed, the investigators demonstrate a marked difference in expression of growth factor, cell adhesion, and chemotraction genes in CD31+ versus CD31− cells as well as demonstrating CD31+ cellular formation of tubules with prolonged culture.

Two aspects of this study are of particular importance for potential clinical translation. First, it demonstrates the ease by which a therapeutically relevant cell population can be obtained with the minimum of manipulation. A peripheral blood draw followed by generation of the appropriate cell fraction via automated methods and readministration by percutaneous injection could be accomplished in any number of facilities, at relatively low cost, and with minimal disruption to existing infrastructure. Second, whereas it remains to be seen whether the number of cells generated would be sufficient for therapeutic effect, the abundance of the CD31+ cell population in peripheral blood is certainly a strength. If future head-to-head studies indicated that such a cell population was equally efficacious as other leading contenders in the cell arena derived from peripheral mobilization or adipose tissue or bone marrow, this would further strengthen the case for CD31 selection of PBMCs. Thus, studies like this one by Kim et al. (7) add to the environment of rapid translation for cell-based therapies in ischemic cardiovascular disease.

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