Although algorithms such as the Framingham Risk Score have been instrumental in helping to stratify cardiovascular disease (CVD) susceptibility, it is estimated that 15% to 20% of patients presenting with myocardial infarction (MI) may lack any history of traditional risk factors. These and other observations have prompted investigators to develop additional biological and genetic assays that might improve risk prediction or capture risks that might be orthogonal to well-known and traditional factors such as diabetes, smoking, hypercholesterolemia, and hypertension. One promising but confusing area appears to be the interrogation of circulating cells that have been deemed endothelial progenitor cells (EPCs). In some studies, the levels of these circulating cells appear to provide predictive power about vascular function in healthy people2,3 and future cardiovascular events in patients with disease.4,5 It is tempting to speculate that the quantitative or functional assessment of cells in the circulation with angiogenic or vascular reparative properties might eventually provide a useful biological measurement that could aid in risk assessment. Yet, significant questions remain regarding the true nature of EPCs and the actual role of these cells in disease progression.6 Answers to these and related questions are therefore urgently needed before the tantalizing promise of EPCs can be fully incorporated into any assessment of CVD risk.

The current report by Shaw et al. in this issue of Circulation: Cardiovascular Genetics provides an elegant blueprint of how a combined biological and genetic approach might aid in our efforts to better understand EPC biology and ultimately better define and understand CVD risk. The current study analyzed nearly 1800 participants in the Framingham Heart Study. For the most part, these individuals were healthy and free of overt CVD. The authors performed a simple biological assay to determine the amount of early-outgrowth colony-forming units (CFUs) present in a fasting blood sample. The details of these CFUs will be discussed in more detail later; however, previous work by this group and others has linked a decline in CFUs with increased risk of CVD.2,4 In the present study, the authors again found a significant, albeit relatively modest, association between CFUs and calculated Framingham Risk Score. In an effort to expand our understanding of this phenomenon, the authors next performed a genome-wide association analysis to assess whether genetic polymorphisms might underlie the observed variations in CFU number. Two regions were subsequently identified as being associated with regulating CFU number in this population. These regions include the MOSC1 and the SLC22A3-LPAL2-LPA locus. Interestingly, the SLC22A3-LPAL2-LPA locus had been previously associated with MI risk. Furthermore, in the current analysis, the variant in this locus was associated with low CFU number (P=4.9×10⁻⁷) and increased MI risk (P=1.1×10⁻⁴). These observations therefore provide genetic support for the relationship made previously between CFU number and future cardiovascular events. Furthermore, they suggest that a decline in the regenerative capacity or number of progenitor cells might provide a biological mechanism for the steep age-dependent rise in cardiovascular disease.

These current results build on a large literature attempting to link subtypes of circulating cells to either subclinical disease or overt cardiovascular events. Such analysis has included genetic profiling of circulating monocytes in patients with and patients without disease. Similarly, there is evidence that certain subtypes of monocytes (CD14⁺CD16⁻) might expand in patients with CVD. The majority of work regarding circulating cells and CVD risk has, however, centered on EPCs. Originally isolated by Asahara et al., circulating EPCs were envisioned to be rare circulating cells of bone marrow origin that could form tube-like structures in culture and could be incorporated into vessels within areas of ischemia. These cells appeared to be enriched in the CD34⁺ and Flk-1⁺ fraction of circulating mononuclear cells. After being plated on fibronectin-coated dishes, these presumably more primitive circulating cells appeared to manifest endothelial properties including the VEGF-stimulated release of nitric oxide, the uptake of both lectin Ulex Europeaus agglutinin-1 (UEA-1), and modified low-density lipoprotein, as well as the surface expression of a number of specific endothelial surface markers. This in vitro and in vivo phenotype led the authors to conclude that these cells represented a circulating endothelial progenitor population that might underpin the capacity for vasculogenesis in the adult. Subsequently, many laboratories have studied the biology and relevance of EPCs in a wide range of conditions. Two major approaches have been used to quantify the number of these cells. The first takes advantage of cell surface markers and analyzes levels of presumptive EPC on the basis of a combination of epitopes including CD34, AC133, and KDR.
(Flk-1 in rodents). One advantage of this technique is that it is quick and quantitative. On the other hand, the sole readout of a number can be misleading. As an example, it is important to note that for hematopoietic stem cells, quantification of number and function may give discordant results. For instance, in many rodent models, the number of hematopoietic stem cells, defined by cell surface epitopes, stays constant or actually increases with age. However, the activity of these hematopoietic stem cells (on a per-cell basis) clearly declines as the animal ages.14

The second approach to quantifying EPCs involves colony-forming assays. Since the work of Asahara et al was performed, these colony-forming assays have usually included a preplating step on fibronectin for either 1 day15 or 2 days2 to remove monocytes and mature endothelial cells. Nonadherent cells remaining after the preplating step were subsequently cultured on fibronectin, and colony number was assessed 7 to 10 days later. These approaches were potentially more biological in that they required colony formation, a more functional output than abundance by flow cytometry. Nonetheless, they are labor-intensive and prone to experimental variation, based on a number of circumstances including culture conditions.

Using one or sometimes both of these approaches, there have been literally hundreds of studies correlating EPC number with specific clinical outcomes.6,16 Although EPCs were initially conceived as potential cellular elements contributing to vasculogenesis, our observations that in the absence of overt disease, a decline in EPC numbers correlated with increased CVD risk, led us to speculate that EPCs may be more important in repair of existing vessels rather than in the formation of new blood vessels.2 Although these studies are intriguing, significant questions have arisen. Indeed, in the absence of a true definition of an EPC, different groups are probably measuring different cell populations. Similarly, even with the use of colony-based approaches, variations on how the assay is performed has led to order-of-magnitude variations between different groups with regard to reported abundance of EPCs in healthy people.2,17 This suggests that what one group calls or isolates as a colony is not necessarily the same as another group definition of the same presumed activity. More importantly, concerns have also been raised that using flow-based cell surface identification or using colony-based approaches, the cell being quantified is of hematopoietic, not endothelial, origin.6 These concerns lead to a series of additional important issues. Included among these are: Do the 2 assay systems identify the same or different cell populations? Do the cells identified actually contribute to disease? If they do contribute, is this through the direct repair of existing blood vessels, through the ability to create new vessels, or do they alter disease progression by acting in an indirect paracrine fashion?

These are difficult questions to address through the use of a strictly correlative approach of quantifying EPC number and assessing the presence or absence of a disease phenotype. In contrast, the article by Shaw et al provides a potential strategy to begin to get a handle on these perplexing issues. For instance, if the putative cell identified by flow cytometry or colony assay formation actually contributes to CVD risk, one would predict that genetic factors controlling EPC abundance could also be identified independently in nonbiased genome-wide association studies for CVD. As mentioned, the current study finds this to be the case. Because the SLC22A3-LPAL2-LPA locus previously identified to affect MI risk also appears to regulate CFU number, these observations provide some assurance that the cells identified by colony formation are not biomarkers or epiphenomena but rather potentially intrinsic regulators of atherosclerosis progression. Further analysis regarding which specific gene within this cluster of 3 separate genes in the SLC22A3-LPAL2-LPA locus is actually linked to MI susceptibility might give clues as to how a decline in CFUs contributes to disease. Similarly, genetic interrogation of these cells might be useful in determining whether these 2 isolation approaches are actually measuring similar activities, be it endothelial progenitor activity or some additional property relevant for CVD. Part of this answer can presumably come from analyzing how well these 2 isolation protocols track in healthy or disease populations. The record is mixed. For instance, in some studies in which investigators have simultaneously executed both strategies, the 2 approaches positively correlate with each other and both provide independent measurements of risk.4 In contrast, other studies have suggested that in a given population, one method yields a tighter correlation with disease risk.8 Finally, it has also been noted that in some populations, neither assay has been shown to be especially helpful in delineating risk.15 In this context, a genetic determination of loci that determine abundance should be helpful in identifying whether cells isolated by flow cytometry and colony formation are biologically similar or distinct. For instance, further analysis of genetic factors determining the abundance of CD34+ AC133−KDR+ cells can presumably be performed and compared with those factors regulating CFU number that were described in the current study. Whether the identified loci overlap or are nonconcordant will be instructive.

In summary, the article by Shaw et al opens up a new genetic-based approach to a well-studied but still frustratingly ambiguous area of investigation. The sheer number of previous studies linking various circulating cells to CVD suggests that a biologically important relationship might exist. Yet, to move beyond interesting correlations, it is essential to begin to understand why the number of given cell type varies within a population and to understand how a decline or expansion of a certain cell type might contribute to a disease phenotype. The current article provides a way to move forward and to fulfill the tantalizing promise of EPC biology and CVD risk.

Disclosures

None.

References


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