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Breakthroughs and Views

Characterization of endothelial progenitor cells

Gian Paolo Fadini *, Carlo Agostini, Angelo Avogaro

Department of Clinical and Experimental Medicine, University of Padova, School of Medicine, Italy

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The work by Walenta et al. [1] addressed the relevant problem of endothelial progenitor cell (EPC) characterization in vitro, performing the study of a wide panel of surface antigens.

Given the increasing interest in EPC biology and its potential use as a therapeutic tool for cardiovascular diseases [2,3], this field of investigation is topical and deserves an especial attention.

Previous works have pointed out that methodological problems arise when trying to isolate and culture human EPC from peripheral blood [4,5]. The early method used for the first time by Asahara et al. [6] in their pioneer research aimed at the phenotypical recognition of endothelial cells derived from peripheral blood mononuclear cells (PBMC). Basically it was based on two well-known functions of endothelial cells: the uptake of low density lipoproteins and the binding of lectin. The rationale underlying many studies thereafter was that the more endothelial cells you obtain from the culture of a subject's PBMC, the more EPC should circulate in his peripheral blood [7,8]. That the isolated cells are actually the endothelial progenitors should be held with caution. Indeed, by definition EPC must retain progenitor cell characteristics, such as ability of long-term replication and stemness properties [9]. More recent papers have clarified that PBMC-derived endothelial cells represent a mixed population including mature circulating endothelial cells, shed off the vessel wall, and monocyte/macrophage-derived cells assuming endothelial phenotype in culture [4]. This is attested by the observation that also $CD14^+$ cells can differentiate toward the endothelial lineage in appropriate culture conditions [10–12]. Among this heterogeneity, only a small proportion is represented by true endothelial progenitors. Nonetheless, the current belief is that true EPC can be isolated by means of exploiting their outgrowing capacity, thanks to which one is licensed to call "EPC" only the endothelial cells that survive and proliferate two weeks after PBMC plating [5].

In the work by Walenta et al., cells were cultured for up to 10 days and were characterized in terms of LDL uptake, lectin binding, and surface antigen at flow cytometry. Therefore, it is not surprising that an extreme heterogeneity characterizes PBMC-derived endothelial-like cells in terms of surface antigens. What appears unlikely is that most (90%) of the culture endothelial cells are positive for both LDL uptake and lectin staining, while only an extreme minority is positive for VEGF-R2 and CD31 at flow cytometry. Flow cytometry data presented in the paper indicate that a majority (24.86%) of $LDL^+Lectin^+$ cells at 10 days are T lymphocytes, while only 2.35% are positive for endothelial markers (unfortunately, the percentage of cells positive for CD146 only, that is the elective marker of mature endothelial cells, is not reported). These data do not correspond to results from other studies, which revealed up to 75% of positivity to endothelial markers [13]. It is also quite strange because T lymphocytes should not share endothelial properties. Rather, it was expected heterogeneity be due to monocyte contamination, given that they can assume endothelial shape and may share antigens and function of the endothelium; moreover, macrophages in the plaque uptake LDL to become foam cells.

Another unclear point is that the sum of CD34, CD133, VEGF-R2, CD31, CD3, CD14, and CD45-positive cells accounts for about 66% at time zero and falls to 31% after 10 days of culture, even after correcting for dual and triple positivity. This leads to the paradoxical conclusion that more than 60% of $LDL^+Lectin^+$ cells are not lymphocytes, nor macrophages, nor endothelial cells. Yet, their phenotype remains unresolved.

* Corresponding author.
E-mail address: gianpaolofadini@hotmail.com (G.P. Fadini).

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These strange results may depend upon technical biases. In order to perform flow cytometry, cultured cells must be detached and exposed to treatments that may alter surface antigens (for example, trypsin may cleave membrane proteins). To exclude such methodological problems, data from flow cytometry should be compared with data obtained by in situ immunofluorescence. Previous works have shown that the majority of $LDL^+Lectin^+$ cells are positive for VEGF-R2, CD31, and vWF at IF [14].

In conclusion, the work by Walenta confirms once more the heterogeneic characteristics of PBMC-derived endothelial cells, but, upon appropriate evaluation, they appear to suffer from some methodological problems that preclude its clear interpretation. To have definitive data on EPC characteristics in vitro investigators should prolong cultures beyond two weeks, avoid trypsin use, and perform parallel immunofluorescence.

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