

Endothelial Progenitor Cells in Mixed Cryoglobulinemia

FRANCESCO MARCHI, DANIELE CHIMENTI, ANTONIO TAVONI, ALESSANDRA DELLA ROSSA, STEFANO BOMBARDIERI, and PAOLA MIGLIORINI

ABSTRACT. *Objective.* Endothelial progenitor cells (EPC) are a rare population of circulating cells involved in vascular homeostasis. Our aim was to analyze EPC in patients with mixed cryoglobulinemia (MC). *Methods.* EPC were evaluated by cytometry according to guidelines of the International Society for Hematotherapy and Graft Engineering in 17 patients with MC and 36 controls. *Results.* Numbers of EPC were significantly increased in MC compared to controls and correlated with cryocrit, but not with clinical manifestations of the disease. *Conclusion.* The high number of EPC might indicate an intact vascular repair ability. Alternatively, EPC might be defective in homing ability and their increase may represent the attempt to restore vascular integrity. (J Rheumatol First Release June 1 2010; doi:10.3899/jrheum.100012)

Key Indexing Terms:

ENDOTHELIAL PROGENITOR CELLS MIXED CRYOGLOBULINEMIA VASCULITIS

The peripheral blood of adults contains a small population of bone marrow-derived endothelial progenitor cells (EPC) that are able to differentiate into mature endothelial cells and thus are involved in endothelial repair and vasculogenesis¹. In patients with coronary artery disease, numbers of EPC are inversely correlated with cardiovascular risk factors², and this has an important prognostic value, that is, to predict the occurrence of cardiovascular events and death from cardiovascular causes³. EPC have been shown to home to sites of vascular damage and to contribute to the revascularization of ischemic areas⁴.

A role of EPC in vascular repair in patients with vasculitis has also been proposed, but information on this is limited. In antineutrophil cytoplasmic antibody-associated vasculitis⁵ and in Kawasaki disease⁶ EPC are significantly increased in patients that, after immunosuppressive therapy, have low disease activity.

We analyzed levels of EPC by flow cytometry in a group of patients with mixed cryoglobulinemia (MC).

MATERIALS AND METHODS

Patients. Seventeen patients (12 women, 5 men, mean age 72 yrs, range 47–85 yrs) with MC followed in the clinical immunology and rheumatology units were recruited consecutively. MC was diagnosed in the presence of Meltzer's triad (purpura, weakness, and arthralgia) and cryoglobulins in the sera. A full clinical and serological evaluation was performed in all

patients, including measurement of complement and cryocrit (percentage of packed cryoglobulins after centrifugation of serum at 4°C).

Thirty-six healthy laboratory personnel (26 women, 10 men, mean age 40 yrs, range 20–56 yrs) served as controls.

Detection of EPC. EPC were assessed by flow cytometry in EDTA-pre-served whole blood^{7–9}. Briefly, the blood sample was stained within 2 h after collection by anti-CD34 PerCP-Cy5.5 (Becton Dickinson, Franklin Lakes, NJ, USA); anti-VEGFR2-FITC (R&D Systems, Minneapolis, MN, USA); and anti-CD133-PE (Miltenyi Biotec, Bergisch Gladbach, Germany). After 30 min, erythrocytes were lysed by ammonium chloride and the samples were run in the flow cytometer.

In parallel samples, whole blood (0.1 ml) was added to a tube containing a predefined number of fluorescent beads (Trucount, Becton Dickinson), stained for 30 min with anti-CD34-PE and anti-CD45-FITC (Becton Dickinson), and run in the flow cytometer after erythrocyte lysis. Following modified guidelines of the International Society for Hematotherapy and Graft Engineering (ISHAGE)^{9,10}, the use of defined numbers of fluorospheres allowed measurement of the absolute number of CD34+ cells. The number of EPC was then obtained by calculating the percentage of CD34+ cells that coexpressed CD133 and VEGFR2.

Nonparametric assays were used for statistical analysis (Mann-Whitney test and Spearman correlation coefficient as appropriate).

RESULTS

MC was associated with hepatitis C virus in 16 patients and hepatitis B virus in 1 patient. C4 levels were low in 16 patients and in 8 of them C3 was also reduced. Thirteen patients had liver involvement and 3 developed cirrhosis; renal involvement was diagnosed in 4 patients. Eight patients had sicca syndrome and 3 Raynaud's phenomenon. Nine patients were treated with low-dosage steroids (< 10 mg prednisone equivalent), in 1 associated with mycophenolate mofetil; 11 patients received antihypertensive drugs (Ca²⁺ channel blockers, angiotensin-converting enzyme inhibitors) that maintained blood pressure in the normal range.

From the Clinical Immunology Unit, Department of Internal Medicine, University of Pisa, Pisa, Italy.

F. Marchi, MD, PhD; D. Chimenti, MSc; A. Tavoni, MD; A. Della Rossa, MD; S. Bombardieri, MD; P. Migliorini, MD.

Address correspondence to Dr. P. Migliorini, Clinical Immunology Unit, Department of Internal Medicine, University of Pisa, Via Roma 67, 56126 Pisa, Italy. E-mail: p.migliorini@med.unipi.it

Accepted for publication February 9, 2010.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2010. All rights reserved.

Levels of EPC were significantly increased in patients with MC compared to controls (median value 84 vs 52 cells/ml; $p = 0.032$; Figure 1). A direct correlation was observed with cryocrit ($p = 0.02$; Figure 2), but not with levels of complement. EPC levels did not differ in subgroups of patients with different types of organ involvement or in patients treated with steroids compared to untreated patients, and were not related to disease duration or to the presence of active vasculitis.

In particular, the 4 MC patients displaying the highest numbers of EPC were not homogeneous in terms of treatment, disease phenotype, or disease activity, and they did not differ significantly from the remaining patients.

DISCUSSION

Our findings indicate that patients with MC were characterized by high numbers of EPC, which were correlated with

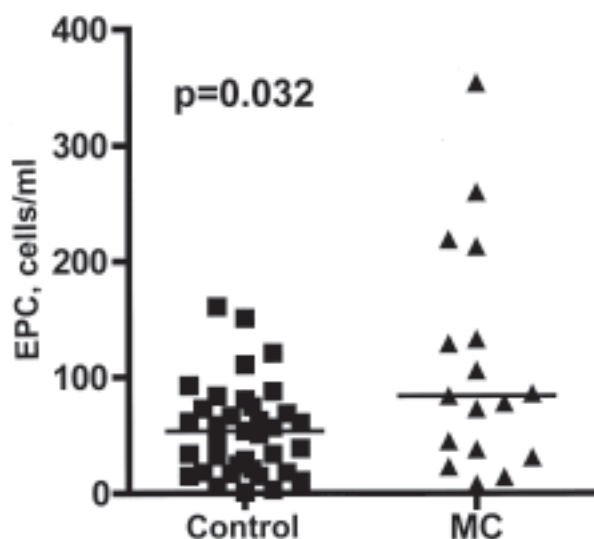


Figure 1. Number of EPC/ml blood was measured in controls and MC patients; number of EPC was significantly higher in MC patients (Mann-Whitney test).

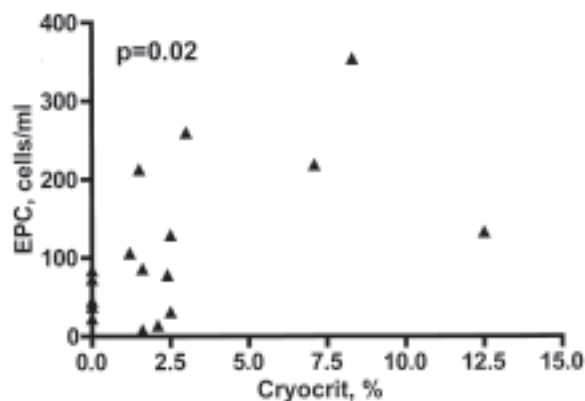


Figure 2. Positive correlation between cryocrit (percentage of packed cryoglobulins) and EPC level in MC patients (Spearman rank correlation test).

cryocrit data and were not related to other serological or clinical disease measures.

Similar results have been reported in other small and large-vessel vasculitides^{5,6}, but comparison with data obtained from patients with other disorders is difficult. Different methods for evaluation of EPC are currently employed, which probably detect distinct subsets of endothelial cell and thus are not highly correlated¹¹. EPC can be detected by functional assays, based on their capacity to form endothelial cell colonies, or by phenotypic characterization, taking advantage of membrane antigen expression. In patients with vasculitis, Nakatani, *et al*⁶ detected EPC as CD133-positive cells out of isolated endothelial cells, whereas de Groot, *et al*⁵ performed an *in vitro* functional assay.

In our study, the first report on numbers of EPC in MC, we followed the ISHAGE guidelines for enumeration of stem cells; using a known amount of fluorescent microbeads and flow cytometry, we determined an absolute amount of CD34+ cells coexpressing VEGFR2 and CD133. This combination of surface markers is widely accepted for identification of “true” EPC in humans; but recent data suggest that isolated CD34+ VEGFR2+ CD133+ cells do not generate new endothelial cells, but instead represent hematopoietic-derived cells that indirectly contribute to vascular homeostasis¹².

In patients with MC, high numbers of EPC are observed despite long disease duration and advanced age, conditions usually associated with a decline of EPC^{13,14}. In this respect, it is important to stress that the control group we studied had a lower mean age than the MC group (40 vs 72 yrs): thus, a “high” number of EPC was expected in the controls.

The correlation of number of EPC with amount of cryoglobulin is an interesting and unexpected finding: cryocrit, in fact, is not related to the activity or severity of the disease. This correlation may suggest a more extensive endothelial damage directly mediated by cryoglobulins, or due to hyperviscosity that may induce a higher bone marrow output of EPC; alternatively, higher amounts of cryoglobulins may interfere with the homing of EPC and thus increase their amount in the circulation.

Thus, the high numbers of EPC might indicate an intact vascular repair ability or represent an attempt to counteract a defect in homing. Followup studies have been planned to clarify whether the number of EPC changes with disease activity, and functional assays will be performed to verify the homing ability of EPC in patients with MC.

REFERENCES

1. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;275:964-7.
2. Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, et al. Number and migratory activity of circulating endothelial

- progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001;89:E1–7.
3. Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, et al. Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl J Med* 2005;353:999-1007.
 4. Zampetaki A, Kirton JP, Xu Q. Vascular repair by endothelial progenitor cells. *Cardiovasc Res* 2008;78:413–21.
 5. de Groot K, Goldberg C, Bahlmann FH, Woywodt A, Haller H, Fliser D, et al. Vascular endothelial damage and repair in antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum* 2007;56:3847-53.
 6. Nakatani K, Takeshita S, Tsujimoto H, Kawamura Y, Tokutomi T, Sekine I. Circulating endothelial cells in Kawasaki disease. *Clin Exp Immunol* 2003;131:536–40.
 7. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood* 2000;95:952-8.
 8. Khan SS, Solomon MA, McCoy JP Jr. Detection of circulating endothelial cells and endothelial progenitor cells by flow cytometry. *Cytometry B Clin Cytom* 2005;64:1-8.
 9. Keeney M, Chin-Yee I, Weir K, Popma J, Nayar R, Sutherland DR. Single platform flow cytometric absolute CD34+ cell counts based on the ISHAGE guidelines. *Cytometry* 1998;34:61-70.
 10. Brocklebank AM, Sparrow RL. Enumeration of CD34+ cells in cord blood: a variation on a single-platform flow cytometric method based on the ISHAGE gating strategy. *Cytometry* 2001;46:254-61.
 11. Van Craenenbroek EMF, Conraads VMA, Van Bockstaele DR, Haine SE, Vermeulen K, Van Tendeloo VF, et al. Quantification of circulating endothelial progenitor cells: a methodological comparison of six flow cytometric approaches. *J Immunol Methods* 2008;332:31-40.
 12. Timmermans F, Plum J, Yoder MC, Ingram DA, Vandekerckhove B, Case J. Endothelial progenitor cells: identity defined? *J Cell Mol Med* 2009;13:87-102.
 13. Fadini GP, Tognon S, Rodriguez L, Boscaro E, Baesso I, Avogaro A, et al. Low levels of endothelial progenitor cells correlate with disease duration and activity in patients with Behcet's disease. *Clin Exp Rheumatol* 2009;27:814-21.
 14. Jie KE, Goossens MH, van Oostrom O, Lilien MR, Verhaar MC. Circulating endothelial progenitor cell levels are higher during childhood than in adult life. *Atherosclerosis* 2009;202:345-7.