CD41 expression is associated with the earliest stages of mouse hematopoiesis. It is notably expressed on some cells of the intra-aortic hematopoietic clusters, an area where the first adult-repopulating hematopoietic stem cells (HSCs) are generated. Although it is generally accepted that CD41 expression marks the onset of primitive/definitive hematopoiesis, there are few published data concerning its expression on HSCs. It is as yet uncertain whether HSCs express CD41 throughout development, and if so, to what level. We performed a complete in vivo transplantation analysis with yolk sac, aorta, placenta, and fetal liver cells, sorted based on CD41 expression level. Our data show that the earliest emerging HSCs in the aorta express CD41 in a time-dependent manner. In contrast, placenta and liver HSCs are CD41-.... Thus, differential and temporal expression of CD41 by HSCs in the distinct hematopoietic territories suggests a developmental/dynamic regulation of this marker throughout development. (Blood. 2011;117(19):5088-5091)
reaction (PCR). Signal quantitation was by DNA normalization (myogenin) and Ly6A GFP control DNA dilutions. For multilineage repopulation analysis, T, B, erythroid, and myeloid cells were sorted from recipient bone marrow (BM) and spleens after antibody staining (supplemental Table 1). Primary recipient BM (2 x 10^6) cells were injected into secondary irradiated recipients to assess self-renewal capacity. For the Ly5.2 recipients injected with Ly5.1 cells, percentage chimerism was determined by flow cytometric analysis, T, B, erythroid, and myeloid cells were sorted from recipient bone marrow (BM) and spleens after antibody staining (supplemental Table 1). Primary recipient BM (2 x 10^6) cells were injected into secondary irradiated recipients to assess self-renewal capacity. For the Ly5.2 recipients injected with Ly5.1 cells, percentage chimerism was determined by flow cytometric analysis, T, B, erythroid, and myeloid cells were sorted from recipient bone marrow (BM) and spleens after antibody staining (supplemental Table 1). Primary recipient BM (2 x 10^6) cells were injected into secondary irradiated recipients to assess self-renewal capacity. For the Ly5.2 recipients injected with Ly5.1 cells, percentage chimerism was determined by flow cytometry on blood after erythrocyte lysis (Beckman Coulter) and antibody staining (supplemental Table 1).

Immunostaining

E11 Ly6A GFP embryos were fixed (2% paraformaldehyde/PBS, 4°C, 1-2 hours), cryoprotected (30% sucrose/PBS, 4°C overnight), Tissue Tek embedded, frozen (dry ice), and cryosectioned. Immunohistochemical staining was as described^{17} with anti-CD41 purified, anti-rat IgG1 biotin, and streptavidin-Cy5 (supplemental Table 1) and detected by laser scanning microscopy.

Results and discussion

Flow cytometric analysis of CD41 expression was performed on cells from E11 and E12 AGM and YS, E12 placenta, and E14 FL. Time points correspond to organ-specific peaks of HSC activity. In all tissues, AGM (Figure 1A,H), YS (Figure 1E), placenta (Figure 2A), and FL (Figure 2E), 3 distinct cell populations were observed: CD41^-CD41intermediate (int) and CD41high, in agreement with YS and AGM data.\(^7\) The highest percentage of CD41int cells is found in placenta, compared with AGM, YS, and FL. This is consistent with the high number of hematopoietic progenitors and HSCs in E12 placenta compared with the other tissues as previously described\(^{15,27,28}\) and the high proportion of megakaryocytic lineage (CD41high) cells as determined by coexpression of Gp1bβ (not shown). In all tissues, the frequency of CD41high cells is greater than that of CD41high cells.

To test which CD41 fractions contain hematopoietic progenitors, sorted cells were plated in methylcellulose and colonies counted. Hematopoietic progenitors were found almost exclusively in the CD41int fraction of E11 AGM and YS cells (Figure 2B,F). Although the majority of E12 placenta and E14 FL hematopoietic progenitors were present in the CD41int fractions, some were also CD41^- (Figure 2B,F). Together, these data suggest that CD41 expression on hematopoietic progenitors is developmentally regulated and/or dependent on the specific tissue microenvironment.
To examine whether HSCs during ontogeny express CD41, each sorted cell fraction from the distinct embryonic tissues was transplanted and donor cell engraftment examined 4 months after transplantation. As expected, no mice were reconstituted with the CD41<sup>high</sup> fraction. E11 AGM HSCs were found exclusively in the CD41<sup>int</sup> fraction (Figure 1C). These data indicate, together with previous live imaging data, that CD41 is a marker for the earliest emerging aortic HSCs as they are transiting from endothelial to hematopoietic fate. In contrast, all E12 AGM HSCs were restricted to the CD41<sup>int</sup> fraction (Figure 1J). Thus, CD41 expression on HSCs is not AGM restricted because, when sorted E11 (or E12, not shown) YS cells were transplanted, HSCs were found in both CD41<sup>−</sup> and CD41<sup>int</sup> fractions (Figure 1G). However, in E12 placenta (Figure 2C,H) and E14 FL (Figure 2G,I), HSCs were exclusively in the CD41<sup>−</sup> fraction. The presence of HSCs in the E12.5 placenta CD41<sup>−</sup> fraction was previously reported, but, as stated by the authors, this was probably because of contaminating CD41<sup>−</sup> cells. At E11.5, no repopulation was found with either the CD41<sup>int</sup> or CD41<sup>−</sup> fractions. At E11.5, no repopulation was found with either the CD41<sup>int</sup> or CD41<sup>−</sup> fractions (Figure 1G).

Figure 2. Phenotypic and functional analyses of CD41-sorted cell fractions of embryonic HSC reservoirs. E12 placenta (A-C,H) and E11/E14 liver (D-G,J). (A,E) Flow cytometric analysis of E12 placenta and E14 fetal liver (FL) showing representative sorting gates (red represents CD41<sup>high</sup>; green, CD41<sup>int</sup>; and blue, CD41<sup>−</sup>) and percentages of cells in each fraction. (B,F) In vitro colony-forming unit in culture (CFU-C) analyses showing the total number of hematopoietic progenitors per 1000 cells in each CD41-sorted fraction of E12 placenta and E14 FL cells. Each sample was analyzed in triplicate for each dilution. (C,G) In vivo hematopoietic repopulation analysis of CD41-sorted fractions of E12 placenta and E14 FL 4 months after transplantation. Percentage of repopulated mice showing greater than 10% donor chimerism in peripheral blood is shown. Numbers above columns indicate the number of mice repopulated/number of mice transplanted. Dose of injected cells is indicated as embryo equivalents (ee). n = 5 for E12 placenta, and n = 3 for E14 FL. (D) CD41 immunostaining of E11 Ly6A GFP embryo section showing the liver. (Top panel) Red fluorescent CD41 expression in hematopoietic cells. (Middle panel) Green fluorescent Ly6A GFP expression in hematopoietic cells. (Bottom panel) Merged fluorescence. The lack of yellow fluorescence indicates no coexpression of CD41 and Ly6A in liver hematopoietic cells. Image acquisition was from LSM510NLO/FCS confocal microscope (Carl Zeiss BV) with 40×1.3 NA water objective and Vectashield medium (Vector Laboratories). LSM image software was used (Carl Zeiss BV). (H-J) Representative semiquantitative PCR analysis of hematopoietic tissue DNA 4 months after transplantation from (H upper panel) a primary recipient injected with 2 ee of E12 placenta CD41<sup>−</sup> cells (l upper panel) 0.1 ee of E14 FL. Representative semiquantitative PCR analysis of peripheral blood DNA from 6 secondary recipients injected with BM cells from the primary E12 placenta CD41<sup>−</sup> recipient (H lower panel) and 5 recipients injected with BM cells from the primary E14 FL CD41<sup>−</sup> recipient (I lower panel). Donor indicates the human β-globin PCR fragment, and Myo indicates the myogenin DNA normalization control PCR fragment. DNA dilution controls (0%-100%) were used to quantitate percentages of donor chimerism that are indicated below each lane. PB indicates peripheral blood; Th, thymus; LN, lymph node; Sp, spleen; M, myeloid (sorted from BM); E, erythroid (sorted from BM); L, lymphoid (sorted from BM); B, B lymphoid (sorted from spleen); and T, T lymphoid (sorted from spleen).
CD41 expression. Hence, these data, showing that CD41 is a temporally restricted early HSC marker, are pivotal to future studies of its transcriptional regulation and role in HSC generation/migration.

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References


18. de Bruijn MF, Speck NA, Peeters MC, Dzierzak E. Definitive hematopoietic stem cells first develop within the major arterial regions of the mouse embryo. EMBO J. 2000;19(11):2465-2474.


CD41 is developmentally regulated and differentially expressed on mouse hematopoietic stem cells

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