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Intracellular Complement (Complosome) is Expressed in Several Types of Human Adult Bone Marrow—Derived Stem Cells

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For many years, it was accepted that the inactive components of the complement cascade (ComC), including C3 and C5 proteins, were synthesized in the liver and subsequently become released into circulation to be activated in cascade reactions leading to the release of active cleavage fragments including C3a and C5a [1]. G-protein coupled receptors for C3a and C5a known as C3aR, C5aR1, and C5L2 or C5aR2, were detected on several types of embryonic and adult cells. It is well known that this liver-derived complement plays a vital role in tissue development and homeostasis [1]. Nevertheless, the most recent data demonstrating that ComC components are also expressed at the protein level by normal lymphocytes and, moreover, may activate intracellular ComC receptors in an intracrine-dependent manner, put more exciting complicity to this system [2]. This novel intriguing intracellular regulatory loop operating in lymphocytes has been described by Kemper et al. as ''complosome'' and orchestrates several T cell responses and metabolism of these cells [2]. In addition to lymphocytes, intracellular complement elements have been described in many types of cells belonging to innate immunity (e.g., monocytes, granulocytes), bone marrow fibroblasts, and some malignant cells, e.g., in the case of ovarian and colon cancer [2].

Since hematopoiesis and lymphopoiesis have a common hemato/lymphopoietic stem cell origin, and similar mechanisms regulate the biology of these cells [3], in footsteps of this intriguing data, we asked if complosome is also expressed and functional in normal HSPCs. In fact, we reported for the first time that human umbilical cord blood (UCB) purified $CD34^+CD38^-$ cells as well as murine $Sca\text{-}1^+lin^-CD45^+$ cells, which both are enriched for population of hematopoietic stem/progenitor cells (HSPCs) express mRNA for complosome components [4]. Considering that human and murine bone marrow (BM) contains, besides HSPCs, several types of other stem cells, including mesenchymal stromal cells (MSCs), endothelial progenitors (EPCs), and very small embryonic like stem cells (VSELs), as the next step we phenotyped human BM-derived and purified stem cells for expression of mRNA encoding crucial complosome elements.

We employed FACS sorter to purify human BM-derived VSELs ($CD133^+lin^-CD45^-$), HSPCs ($CD34^+Lin^-CD45^+$), EPCs ($CD45^-KDR^+CD133^+$) and MSCs ($CD45^-KDR^+CD133^-CD73^+$). Figure 1A demonstrates our RT-PCR and RQ-PCR data of human BM-purified stem cells expressing C3, C3aR, C5, C5aR1 and C5aR2 (C5L2). Interestingly, as compared to MNCs, all evaluated human BM-derived stem cells express at high-level mRNA for these genes. Therefore, our data indicates that all types of human BM-residing stem cells may be regulated by complosome in an intracrine/paracrine-dependent manner. This expands our knowledge of how early stages of stem cell specification could be governed in a more complex way by potentially involving intrinsic innate immunity signals. Moreover, in our recent work by employing C5-KO and C5aR1-KO animals as a model, we already demonstrated that in fact, the complosome regulates trafficking, proliferation, and metabolism of murine HSPCs [5]. Based on this

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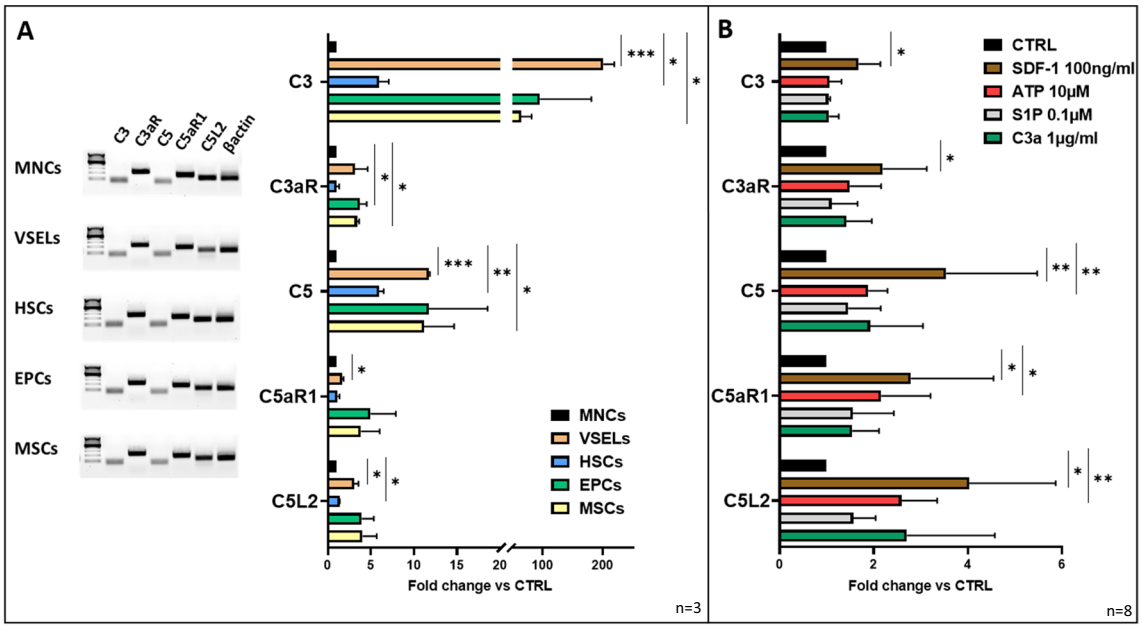

Fig. 1 Complosome is expressed in human bone marrow-derived stem cells. (Panel A) – left—shows RT-PCR expression of mRNA for crucial complosome elements in mononuclear cells (MNCs), very small embryonic like stem cells (VSELs), hematopoietic stem cells (HSCs), endothelial progenitors (EPCs) and mesenchymal stem cells (MSCs). Representative gel is shown. (Panel A)—right—Quantitative RQ-PCR expression data for crucial complosome elements in mononuclear cells (MNCs), very small embryonic like stem cells (VSELs), hematopoietic stem cells (HSCs), endothelial progenitors (EPCs) and mesenchymal stem cells (MSCs) ($n=3$). (Panel B). Stimulation of UCB hematopoietic stem/progenitor cells with chemotactic factors upregulates expression of complosome mRNA. Effect of stimulation of human UCB-derived HSCs with Stromal derived factors-1 (SDF-1), extracellular Adenosine triphosphate (eATP), Sphingosine-1 phosphate (S1P) and C3a ($n=8$). *$p \leq 0.05$, **$p \leq 0.01$, **$p \leq 0.001$. Sequences of primers are available upon request

data, we concluded that complosome is a novel regulator of hematopoiesis, and provided data that its defect in C5-KO mice results in the defective formation of membrane lid rafts (MLRs) required for optimal signaling from so-called "raftophilic receptors" [3–5]. MLRs are microdomains that float freely in the membrane bilayer and are enriched for their functional integrity for cholesterol, sphingolipids, and ceramides [4, 5]. MLRs "raftophilic" receptors include, e.g., the CXCR4 receptor for stromal-derived factor 1 (SDF-1), common β-subunit chain for IL-3, GM-CSF and IL-5 receptors, the c-kit receptor for stem cell factor (SCF), and the VLA-4 integrin receptor, which all together regulate migration, proliferation, and adhesion of HSPCs [4, 5]. Thus, the cell metabolism and migration are coordinated by "raftophilic" receptors. It explains why defect in MLRs formation detected in C5-KO deficient mice has a negative effect on migration of HSPCs [4]. Moreover, a decrease in glucose and amino acid metabolism seen in complosome-deficient C5-KO mice has an adverse impact on metabolism of these cells and their proliferation [4]. We anticipate that the same may occur in human stem cells, and studies involving CRISP-Cas9 strategy to downregulate the expression of C3 and C5 in human stem cells are initiated in our laboratory.

Therefore, based on our data with murine HSPCs showing a role of intracellular complosome in regulating trafficking and metabolism of these cells, we become as next interested if crucial factors involved in stem cell trafficking such as stroma derived factor-1 (SDF-1), extracellular adenosine triphosphate (eATP), and sphingosine-1 phosphate (S1P) may regulate expression of mRNA for complosome elements. Purified human UCB-derived HSPCs were stimulated by these important factors involved in their migration and metabolism, and mRNA level as compared to non-stimulated cells was assessed by RQ-PCR (Fig. 1B). As predicted, we noticed that mRNA for intracellular complosome elements was upregulated in human HSPCs, and the most effective stimulatory factor was SDF-1, which is a ligand for CXCR4 chemokine receptor. The SDF-1-CXCR4 axis is a major homing factor responsible for the retention of HSPCs in BM stem cell niches.

An open question remains still: how complosome become activated in the cytosol? It was known that in the peripheral blood complement becomes activated by three activation pathways, including *i)* classical, *ii)* alternative, or *iii)* mannan-binding lectin pathway [1]. For activation of intracellular complement in lymphocytes, it has been proposed that this process is triggered by cathepsin L [2]. However, recently it has been postulated that intracellular complosome could be activated in other types of cells by cathepsin D. However, we cannot exclude other potential activators,

such as intracellular thrombin that become absorbed from the microenvironment or proteinase 3. To support this, thrombin has C5 convertase activity, and proteinase-3 KO mice have a reduced number of HSPCs in BM. Finally, we cannot exclude the involvement of other proteases and even the simultaneous activation of complementary redundant proteinase pathways.

In conclusion, our data indicates that intracellular innate immunity elements, including complosome, may play an important role in stem cell specification. It also may suggest that experiments using liver-derived complement in tissue/organ regeneration should be reappraised to see how important in these processes intracellularly expressed complosome is.

Authors Contribution MZR – conceived idea and designed experiments. KB – performed experiments and prepared Figure. All the authors provided comments and approved manuscript.

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Data Availability Detailed data are available upon reasonable request.

Declarations

Ethical Approval Human research cells units were obtained from healthy donors. This study was performed following the guidelines and approval of the Medical University of Warsaw Bioethics Committee (permission number KB/3/2018).

Competing Interests None identified.

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