

Inhibiting HDAC for human hematopoietic stem cell expansion

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Commentary

In this issue of the *JCI*, Chaurasia and colleagues report an impressive ex vivo expansion of HSCs from human cord blood (CB) using cytokines and altering epigenetic modifications. The application of this protocol provides information that has potential for clinical consideration. The enhanced expansion of CB HSCs is a substantial advance over recent work from the Chaurasia and Hoffman group, in which ex vivo production of human erythroid progenitor cells from CB was promoted by chromatin modification. Moreover, this study takes advantage of information from the rapidly emerging, but not yet fully elucidated, field of epigenetics.

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regulate the T and B cell responses that effective vaccines seek to generate.

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Inhibiting HDAC for human hematopoietic stem cell expansion

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In this issue of the *JCI*, Chaurasia and colleagues report an impressive ex vivo expansion of HSCs from human cord blood (CB) using cytokines and altering epigenetic modifications. The application of this protocol provides information that has potential for clinical consideration. The enhanced expansion of CB HSCs is a substantial advance over recent work from the Chaurasia and Hoffman group, in which ex vivo production of human erythroid progenitor cells from CB was promoted by chromatin modification. Moreover, this study takes advantage of information from the rapidly emerging, but not yet fully elucidated, field of epigenetics.

Limited HSCs in cord blood prevent broad clinical use

The limited number of HSCs in single cord blood (CB) collections has been problematic for efficient engraftment in adult patients (1), hence the efforts of numerous groups to compensate for low HSC numbers by attempting to transplant double CB units, expand HSCs from CB ex vivo,

increase the homing capabilities of HSCs through priming and other maneuvers, or modulate the recipient's microenvironment for more efficient engraftment (1). A number of efforts have been put forth to expand human HSCs ex vivo (2), with limited success.

HDAC inhibition dramatically improves CB HSC expansion

Epigenetics is a high-profile area of investigation (3). In this issue, Chaurasia et al. (4) followed up on their previous studies (5) by focusing on means to more effectively expand HSC populations isolated from CB using histone deacetylase inhib-

itors (HDACis), primarily valproic acid (VPA), either in the context of cytokine-primed CB cells or with greater effect in addition to cytokines for the 7-day period of ex vivo cell culture (Figure 1). The cytokine cocktail included stem cell factor, FLT3 ligand, thrombopoietin, and IL-3 which, together in the presence of VPA, produced a phenomenal expansion of engraftable HSCs as assayed by state-of-the-art procedures. Chaurasia and colleagues evaluated and quantitated human HSC engraftment and repopulation in sublethally irradiated NOD/SCID IL-2 receptor γ c-null (NSG) mice (4). Using limiting cell dilution analysis, Chaurasia et al. determined that the frequency of SCID-repopulating cells (SRCs) after ex vivo culture of CB cells with cytokines and VPA was 1 SRC in every 31 cells, while CB cells cultured with only cytokines produced 1 SRC in 9,225 cells, and input (unexpanded CB cells) had 1 SRC in 1,115 cells. This translated to respective numbers of 32,258 SRCs from ex vivo-cultured CB cells in the presence of cytokines plus

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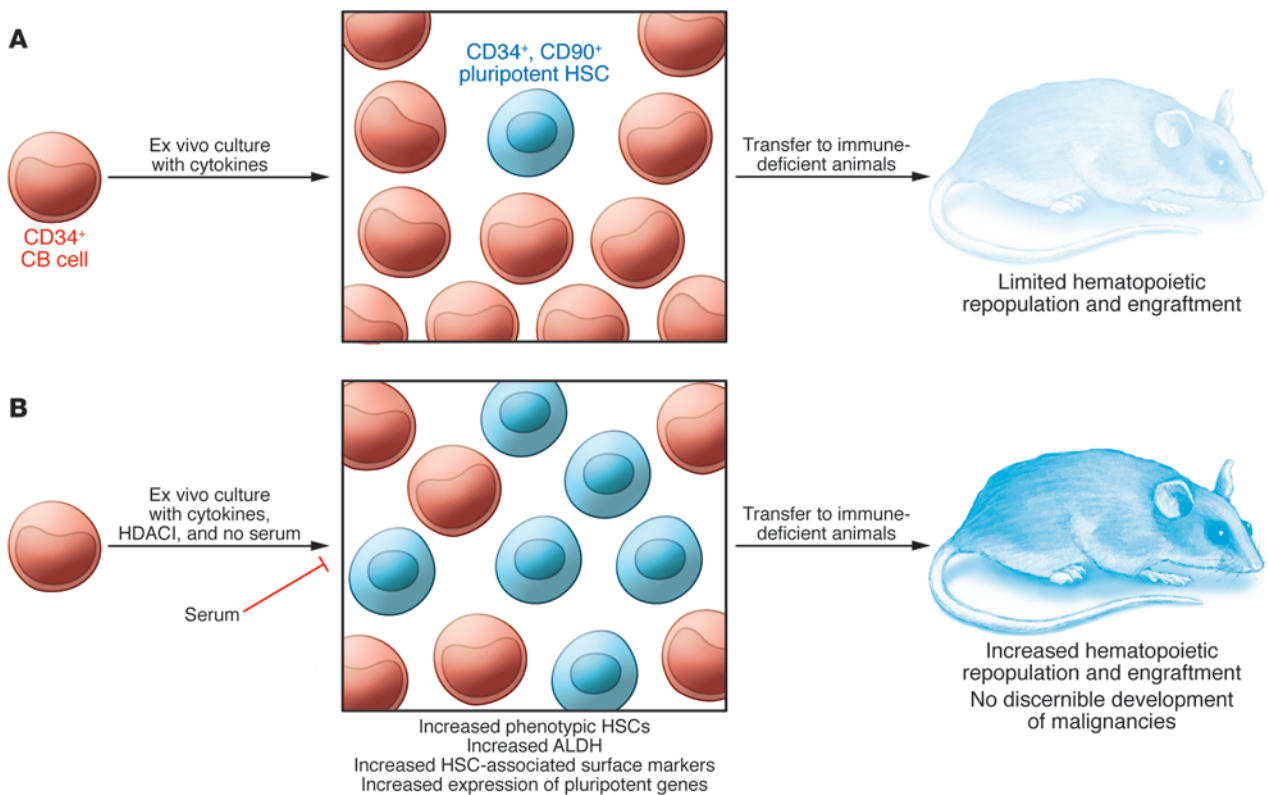


Figure 1

HDAC inhibition improves ex vivo expansion of HSCs from CB. Human CB was collected and exposed for 7 days to a cytokine cocktail or a cytokine cocktail plus HDACi in the presence (A) or absence (B) of serum. CB cells cultured in the presence of cytokines and HDACi exhibited increased numbers of cells expressing HSC phenotypes, including increased production of ALDH, upregulation of HSC surface markers CD90, c-Kit, integrin $\alpha 6$, and CXCR4, and expression of the pluripotent genes *OCT4*, *SOX2*, and *NANOG* (B). Surprisingly, the presence of serum decreased the efficiency of HDACi-associated HSC expansion (A). Cells expanded in the presence of cytokines and HDACi had a much greater capacity to repopulate hematopoietic cell populations and engraft following transplantation into severely immune-deficient animals (B). The study by Chaurasia et al. (4) raises several questions for future consideration: What serum factors block full HSC expansion? What other intracellular factors are involved in epigenetic reprogramming of HSCs? Will other chromatin-remodeling agents and/or small-molecule inducers of pluripotency promote ex vivo expansion of human HSCs alone or in combination with HDACis? Can expanded HSCs with increased pluripotent gene expression be used for more efficient generation of fully programmed iPSCs?

VPA and 108 SRCs from ex vivo CB cells cultured with cytokines alone, while the input CB contained 897 SRCs. VPA-plus-cytokine-expanded cells also had extensive capacity for secondary mouse repopulation, demonstrating their self-renewal activity. Thus, expansion of CB cells in the presence of cytokines alone under the conditions used in the study by Chaurasia and colleagues resulted in a loss of SRCs, while the addition of VPA with the same cytokines resulted in an approximately 36-fold increase in SRCs compared with the number of input SRCs.

The use of VPA plus cytokines also resulted in increased aldehyde dehydrogenase (ALDH) activity as well as expression of CD90, CD117 (c-Kit), CD49f (integrin $\alpha 6$), and CD184 (CXCR4) on the cell surface. The chemokine receptor CXCR4

binds stromal-derived factor 1 (SDF1/CXCL12), which is known to be involved in homing of HSCs (6, 7). Increased expression of CXCR4 on the cytokine-plus-VPA-cultured cells was associated with increased sensitivity of these cells to chemotaxis in vitro in response to SDF1/CXCL12 and increased HSC homing in vivo. One of the disadvantages of using CB for hematopoietic cell transplantation (HCT) is that compared with transferred bone marrow, the time to neutrophil, platelet, and immune cell reconstitution is slower (1), a phenomenon that is also apparent when two CB units are used for HCT; therefore, increased expression of CXCR4 and possibly CD49f on HSCs may allow for decreased time to engraftment. Even if enhanced CXCR4 does not improve engraftment, there are other

means to decrease the engraftment period, including the use of an inhibitor of dipeptidylpeptidase 4 (DPP4). DPP4 selectively truncates a number of proteins by removing the last two amino acids from the N terminus, usually when the penultimate amino acid is an alanine or proline. SDF1/CXCL12 and other cytokines have a DPP4 site, and DPP4-truncated SDF1/CXCL12 is less efficient in chemotaxis and blunts the activity of full-length SDF1/CXCL12 (8, 9). Moreover, inhibition of DPP4 activity with diprotin A or sitagliptin enhances the homing and engrafting capability of HSCs in mouse models (8, 10).

Of practical interest is the observation that ex vivo expansion of CB cells was much more efficient under serum-free conditions compared with that observed in serum-containing cultures. This dis-



crepancy raises the question as to what exactly in the serum was acting to decrease activity for the output of HSCs. Many different cell populations, cell-derived cytokines, and other factors can act as negative regulators of HSCs (6, 7, 11). Furthermore, these molecules can act at low concentrations, especially in combination with other negative regulators. Identification of the factors involved in the down-modulating effects of serum would be informative. If the modulating factors are proteins, it will be especially important to determine whether they are full-length or DPP4-truncated forms.

HDACI-dependent induction of pluripotent genes

An interesting aspect of the study by Chaurasia and colleagues (4) is the observation that VPA induced an endogenous increase in the expression of pluripotent genes, including *SOX2*, *OCT4*, *NANOG*, and *ZIC3*. Furthermore, siRNA-mediated knockdown of *OCT4*, *SOX2*, and *NANOG* in CB cells limited the ex vivo expansion of phenotypic HSCs, verifying the role of these genes in pluripotency. Induction of these pluripotent genes in CB HSCs did not result in any obvious malignancy or promote the formation of teratomas in primary or secondary NSG mice engrafted with the expanded cells. Moreover, injection of pluripotent CB HSCs into sites that have been shown to form teratomas following embryonic stem cell (ESC) injection did not induce teratoma formation. Pluripotent genes are involved in maintaining stem cell states for ESC lines and in reprogramming somatic cells to exhibit an ESC-like state that is inherent in induced pluripotent stem cells (iPSCs), and expression of these genes is usually associated with the ability of ESCs and iPSCs to form teratomas. Of course, the possibility exists that malignancies may manifest, if and when cytokine-plus-VPA-expanded CB HSCs are used for clinical HCT, and should be kept in mind, as it might take a long time for malignant transformation to occur in patients. In the context of patient HCT, there would be a much longer time for malignancies to manifest compared with the year or so that NSG mice are monitored following human cell engraftment. Regardless of whether ex vivo cytokine-plus-VPA-expanded cells are used clinically, we are already the beneficiaries of increased knowledge about the regulation of HSC function. VPA-plus-cytokine treatment

likely affects cell survival, self-renewal, and proliferation of HSCs, as OCT4 promotes survival and pluripotency in murine ESCs (12). Chaurasia et al. noted a physical association between OCT4 and NANOG in cells cultured with VPA plus cytokines (4), adding further data to support the contribution of this network of interacting transcription factors in pluripotency maintenance. Collectively, these data suggest that other genes are involved in the VPA-plus-cytokine-induced effects that promote ex vivo generation and expansion of human HSCs. The more information we gather toward understanding the functional characteristics of these ex vivo-expanded cells, the more comfortable we may be in considering their clinical application for human HCT. Further informative work will be required to determine the metabolic profile of expanded CB HSCs and how they compare with unmanipulated primary HSCs, ESCs, iPSCs, and other pluripotent cells. Moreover, future studies should investigate the roles and activities of mitochondria in these different pluripotent cell types. Based on the role of HDACIs in promoting HSC expansion, a possible group of proteins to investigate includes members of the sirtuin family of deacetylases (13), including SIRT1, which has been linked to NANOG expression, p53 subcellular localization, and mitochondrial function in murine ESCs (14–16), as well as to hematopoietic cell differentiation during embryogenesis and in adult mice (17). These SIRT1-associated effects are especially apparent under stressful conditions, which ex vivo culture of HSCs can certainly be considered. Other potential players that could be involved in chromatin remodeling of HSCs include DEK, a unique protein that is involved in HSC regulation and hematopoietic progenitor cell biology (18).

Of the eight HDACIs tested in the ex vivo system used by Chaurasia and colleagues, three (VPA, scriptaid, and CAY10433) enhanced cytokine-stimulated HSC expansion (4). Understanding why some HDACIs worked and why others were less effective or failed may shed more light on the mechanisms underlying the reprogramming of CB HSCs. Various chemical approaches are being applied to stem cell biology (19), and some of these approaches, alone or in combination with VPA or other HDACIs, may be of value in deciphering how to increase the numbers and/or potency of human HSCs for therapeutic use. Reprogramming of somatic

cells, such as CB CD34⁺ cells, to an iPSC state results in colonies that are morphologically indistinguishable from ESCs; however, some iPSC colonies contain only partially reprogrammed cells (20). In this context, it would be reasonable to determine whether the CB HSCs produced ex vivo in the presence of VPA and cytokines, which already have enhanced expression of OCT4, SOX2, and NANOG, can be more efficiently induced toward fully reprogrammed iPSCs. Information on how to maximize the generation of iPSCs is of great scientific as well as potential practical interest in the context of regenerative medicine.

Conclusions

The study by Chaurasia et al. (4) presents important steps toward further understanding HSC biology and how to potentially manipulate these cells for therapeutic advantage. There have now been over 30,000 CB HCTs performed (1), and the means to enhance the efficacy of this procedure could benefit many patients with malignant and nonmalignant disorders who cannot otherwise find another appropriate source of HLA-matched allogeneic HSCs for HCT.

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Unraveling the relationship between microbial translocation and systemic immune activation in HIV infection

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Chronic immune activation is a key factor in HIV-1 disease progression. The translocation of microbial products from the intestinal lumen into the systemic circulation occurs during HIV-1 infection and is associated closely with immune activation; however, it has not been determined conclusively whether microbial translocation drives immune activation or occurs as a consequence of HIV-1 infection. In an important study in this issue of the *JCI*, Kristoff and colleagues describe the role of microbial translocation in producing immune activation in an animal model of HIV-1 infection, SIV infection of pigtailed macaques. Blocking translocation of intestinal bacterial LPS into the circulation dramatically reduced T cell activation and proliferation, production of proinflammatory cytokines, and plasma SIV RNA levels. This study directly demonstrates that microbial translocation promotes the systemic immune activation associated with HIV-1/SIV infection.

Immune activation and disease progression in HIV-1 infection

In humans, the gut-associated lymphoid tissue (GALT) contains a large number of activated CD4⁺CCR5⁺ T cells, thus serving as a major site of viral replication and CD4⁺ T cell depletion early in the course of HIV-1 infection (1, 2). In contrast to the rapid depletion of intestinal CD4⁺ T cells, the decline of CD4⁺ T cells in peripheral

blood is much slower and less extensive during acute HIV-1 infection, suggesting that the majority of peripheral CD4⁺ T cells are nonpermissive at this stage (2, 3). During the chronic phase of infection, systemic immune activation is very strongly correlated with disease progression (4). Activation of CD4⁺ T cells increases coreceptor expression and renders these cells more susceptible to HIV-1 infection, and these cycles of activation and infection may drive progression of disease to AIDS (5). In contrast, SIV infection in natural host species very rarely progresses to AIDS and does not result in increased levels of immune

activation, despite high levels of virus replication (6, 7). Infection and depletion of CD4⁺ T cells in GALT can be observed in both SIV and HIV-1 infection; however, natural host species infected with SIV do not often develop systemic immune activation, despite loss of GALT CD4⁺ T cells (6, 7), suggesting that factors other than direct infection and mucosal CD4⁺ T cell loss are essential for the increased degree of immune activation seen in HIV-1 infection.

Microbial translocation in HIV-1 infection

Translocation of microbial products from the lumen of the gastrointestinal tract into the circulation often occurs in HIV-1-infected individuals and is closely associated with systemic immune activation. Many studies have shown that HIV-1-infected individuals have elevated plasma levels of LPS and soluble CD14, which is indicative of LPS-induced monocyte and macrophage activation (8). Plasma levels of other microbial products, such as bacterial DNA and flagellin, are also increased in HIV-1-infected individuals compared with healthy controls (8, 9). Furthermore, there is a negative correlation between plasma

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