inactivating factor Va and factor VIIIa. The importance of its cleavage of factor Va at residue Arg506 is illustrated by the role of the factor V Leiden mutation (Arg506Gln) as a risk factor for venous thrombosis. It has been shown recently that cleavage inactivation of factor Va depends not only on the active site of APC but also on basic residues including Lys191, Lys192, Lys193, Arg229, and Arg230.[1,2] These basic residues lie in loops forming a positively charged surface, or exosite, on APC, which helps position the active site over the factor Va residue Arg506.

APC also participates in a nonanticoagulant reaction, cleavage of protease-activated receptor-1 (PAR-1) on the surface of endothelial cells.[3] This reaction requires the endothelial cell protein C receptor (EPCR) and results in an antiapoptotic/neuroprotective cell phenotype.[4] In this issue of *Blood*, Mosnier and colleagues demonstrate that while mutation of the above basic residues of APC dramatically reduces anticoagulant activity, it does not alter proteolysis of PAR-1 and consequent cytoprotective functions. Proximity of APC to its PAR-1 cleavage site therefore is not facilitated by its exosite but solely by its engagement with EPCR.

What is the importance of these findings? First, they illustrate how proteinases can use different mechanisms to achieve specificity. They show that substrate specificity of APC can be exquisitely controlled by fine-tuning its molecular recognition mechanisms. The mutation of the basic residues in surface loops of APC only alters interaction with factor Va and does not alter its active site and those substrates that do not require broader contact for their proteolysis. Second, they have potentially important implications for the future treatment of severe sepsis. While numerous diverse approaches to therapy of severe sepsis, including those based on bacterial toxins, cytokine function, and cell signaling pathways, have produced encouraging results in animal models, all have failed to be clinically effective.[5] In contrast, intervention in the downstream consequences of sepsis using recombinant anticoagulant proteins has been more promising in humans. Of the 3 natural anticoagulants, antithrombin, tissue factor pathway inhibitor, and activated protein C, however, only the latter has been sufficiently decisive in terms of mortality in large clinical trials to obtain regulatory approval for routine clinical use. This has focused interest on the unique aspects of APC function, their cytoprotective effects, and their possible role in protection against sepsis. This interest has been heightened because, despite APC's beneficial effect upon mortality, bleeding remains a concern in APC therapy and may be a factor in slow uptake of this therapy. The paper by Mosnier et al provides a rationale for preparation and evaluation of restricted-specificity APC preparations with enhanced cytoprotective–to–anticoagulant activity ratios. Such products will have to undergo extensive efficacy and safety testing but offer the potential of an improved therapy based upon a solitary success in this difficult area. ■

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IMMUNOBIOLOGY

Comment on Schlecht et al, page 1808

Plasmacytoid dendritic cell: vive le dilettante!

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Whether plasmacytoid dendritic cells (pDCs) are effective in presenting antigens has been a matter of debate. Schlecht and colleagues provide evidence that pDCs can indeed prime CD8 T cells specific for viral antigens.

Plasmacytoid dendritic cells (pDCs) are a rare population morphologically similar to plasma cells that express major histocompatibility complex (MHC) class II and costimulatory molecules, thereby triggering T-cell proliferation and differentiation. Moreover, they secrete large amounts of type I interferon (IFN; IFN-α and IFN-β) when exposed to DNA and RNA viruses. The ability of pDCs to stimulate T cells and secrete type I IFN has raised 3 fundamental questions. (1) Do pDCs present antigens as effectively as classical DCs? While it is generally agreed that pDCs are less efficient than DCs, whether pDCs can prime naive T cells, as DCs do, or just expand memory T cells, as B cells do, remains a matter of debate. (2) Does activation of pDCs affect the quality of antigen-specific T-cell responses? While activated pDCs up-regulate T-cell stimulatory molecules, it has been proposed that immature pDCs may in fact induce regulatory T cells that actively suppress antigen-specific T-cell responses. (3) Does pDC secretion of type I IFN contribute directly or indirectly to antigen presentation?

In this issue of *Blood*, Schlecht and colleagues address these issues using an original and clever approach that involves activating pDCs with different stimuli in vivo, recovering activated pDCs, loading them with antigen in vitro, and adoptively transferring them into mice. Remarkably, pDCs injected intravenously home efficiently to the spleen, making it possible to study their ability to stimulate or inhibit naive and memory T-cell responses. The results of these experiments demonstrate that the antigen-presenting capacity of pDCs is critically dependent on their state of activation and, in particular, the stimuli used to activate them in vivo. Two types of stimuli were evaluated: a cytosine-guanine (CpG) oligonucleotide, which activates pDCs through toll-like receptor (TLR) 9, and heat-inactivated influenza virus, which stimulates pDCs through TLR7. The pDCs activated with CpG can stimulate memory CD8 T cells but not naive CD8 T cells. In contrast, pDCs activated with influenza virus can prime naive CD8 T cells, although less efficiently than DCs. Moreover, T-cell responses primed by pDCs can be

recalled by a second antigenic challenge as efficiently as those primed by classical DCs. Finally, immature pDCs pulsed with a peptide antigen induce neither antigen-specific CD8 T-cell responses nor tolerance to subsequent challenge with the same peptide.

That antigen presentation is induced by influenza virus but not CpG is surprising, as both stimuli activate the same TLR/myeloid differentiation factor 88 (MyD88) signaling pathway. How can this be? One clue to this difference can be found in one of the earliest experiments performed by Schlecht and colleagues, showing that influenza virus triggers IFN-α secretion while CpG does not. Previous studies have shown that IFN-α is unique in its ability to stabilize peptide–MHC class I complexes, possibly by inducing tapasin, which edits peptide loading onto class I by selecting high-affinity versus low-affinity peptides. Thus, secretion of IFN-α by pDCs may not only induce an antiviral state in neighboring cells, protecting them from the cytopathic effect of the virus, but also enable pDCs to selectively present viral antigens by increasing the half-life of MHC class I–viral peptide complexes. In conclusion, despite their original image as dilettantes of antigen presentation, pDCs may be unexpectedly skilled in triggering T-cell responses specific for viral antigens. ■

HEMATOPOIESIS

Comment on Hess et al, page 1648

Vitamin A metabolism and stemness

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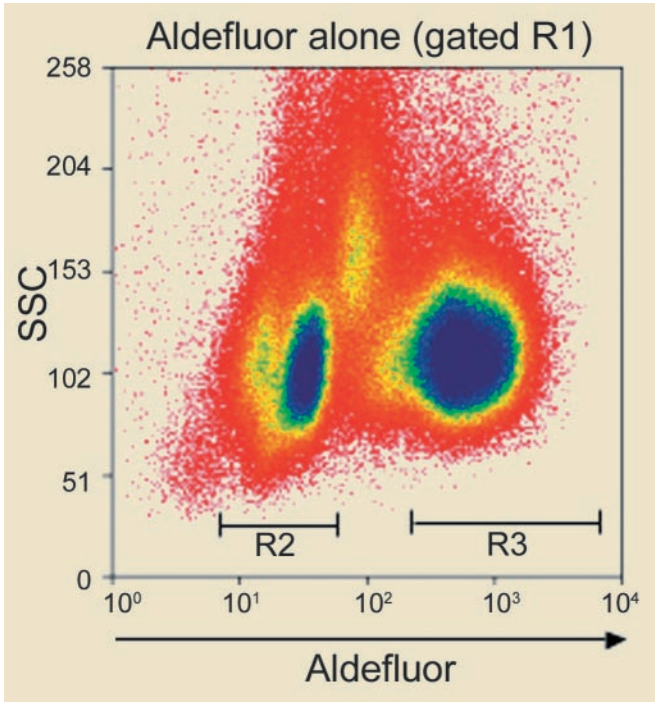
Human hematopoietic stem cells with in vivo multilineage reconstitution potential can be isolated based on their high content of aldehyde dehydrogenase, without the need for recognition of cell surface markers.

Functional properties distinguishing hematopoietic stem cells (HSCs) from other cell types are numerous but very few are suitable for the detection and selection of viable stem cells. In this issue of *Blood,* Hess and colleagues describe the identification and isolation of functional human HSCs based on the expression of cytosolic aldehyde dehydrogenase (ALDH). Although this report does not describe a new distinctive property of HSCs, it offers, for the first time, a detailed and comprehensive characterization of the phenotypic in vitro clonogenic potential and long-term marrow repopulating capacity of lineage-depleted human umbilical cord blood cells fractionated on the basis of their relative expression of ALDH. The use of ALDH to isolate murine and human primitive hematopoietic cells was originally reported several years ago[1,2] and high levels of ALDH expression have been described in stem cells other than those of hematopoietic origin.[3] Cytosolic ALDH is an intracellular enzyme responsible for oxidizing aldehydes. Acetaldehyde, an intermediate in ethanol metabolism, and vitamin A are 2 of its prominent substrates. Metabolic products of vitamin A participate in the regulation of retinoic acid response elements, which are implicated in many mammalian developmental and growth processes. Hess and colleagues took advantage of the oxidizing power of ALDH and used a fluorescent aldehyde substrate, labeled Aldefluor, to isolate a population of umbilical cord blood cells constituting approximately one thousandth of total mononuclear cells contained in this hematopoietic tissue. Depletion of cells expressing lineage markers followed by selection of cells with high ALDH expression (ALDH^{hi}Lin^{-}) yielded a group of cells highly enriched for the expression of surface markers commonly associated with a stem cell phenotype including CD34 and CD133. Interestingly, distinct in vivo biologic functions were recognized for ALDH^{hi}Lin^{-} cells versus those with low expression of ALDH (ALDH^{lo}Lin^{-}), especially when compared in 2 disparate animal models. Fewer ALDH^{hi}Lin^{-} cells were required to engraft nonobese diabetic/severe combined immunodeficiency β2 microglobulin (NOD/SCID β2M) null mice, known to be permissive for the engraftment of more committed cells, than were required to engraft NOD/SCID mice, suggesting that fractionation of HSCs based on the expression of ALDH yielded a mixture of primitive and more committed progenitors among cells expressing high levels of ALDH. However, ALDH^{lo}Lin^{-} cells failed to efficiently engraft in either mouse model, demonstrating that ALDH^{lo}Lin^{-} cells were almost completely devoid of short- and long-term repopulating capacity.

It is not surprising that, although ALDH identified a very small group of hematopoietic cells with reconstitution potential, ALDH^{hi}Lin^{-} cells were demonstrated to be functionally heterogeneous, reflecting, once again, the hierarchical organization of the stem cell pool. Nevertheless, selection of HSCs with ALDH offers a technique based on stem cell function rather than surface phenotype and may have a



Identification and isolation of purified ALDH^{hi}Lin^{-} and ALDH^{lo}Lin^{-} cell populations. See the complete figure in the article beginning on page 1648.