inside blood

Plasma glycolipids levels: new factors regulating the protein C anticoagulant pathway and determining thrombotic risk

Deguchi and colleagues (page 1907) have identified the plasma glucosylceramide (GlcCer), but not other similar glycolipids, as a novel source for supporting the activity of the natural anticoagulant activated protein C (APC). They show that patients below the 10th percentile in GlcCer had an increased incidence of venous thrombosis. Interestingly, subgroup analysis revealed that this relationship only held with the younger patients (younger than 45 years of age). Furthermore, the anticoagulant response to APC correlated with GlcCer concentration in male, but not female, thrombosis patients. These findings raise important clinical and basic questions.

Both the procoagulant and anticoagulant vitamin K–dependent complexes utilize negatively charged phospholipids to assemble the relevant functional complexes. Because the vitamin K domains of the protease components were relatively conserved, it was always assumed that they shared similar lipid determinants. Recently, it has been shown that the phospholipid specificities of the anticoagulant complexes differ markedly from the procoagulant complexes, with phosphatidylethanolamine and lipid oxidation augmenting the anticoagulant responses quite significantly. The present study indicates that plasma glycolipids increase the anticoagulant response to APC markedly. Previous studies by Bertina and colleagues have shown that low anticoagulant responsiveness to APC, regardless of the basis for the poor response, is associated with increased thrombotic risk. The present study may have provided one of the mechanisms responsible for this low response to APC in some of these patients. Another interesting quandary is that platelets support APC activity poorly in vitro, yet in vivo APC has been shown to be a potent inhibitor of thrombin-mediated platelet thrombus formation, suggesting that there are major differences between the platelet dependent functions of APC in vivo and in vitro. In vitro studies with platelet anticoagulant functions are usually done in the absence of plasma. Hence, plasma GlcCer augmentation of APC function may provide one of the explanations for this quandary. Assuming that the clinical linkage between low GlcCer plasma levels and increased risk of thrombosis in young patients is confirmed in additional studies, the results of the present study suggest that there are both age- and sex-related differences that determine the importance of GlcCer in the anticoagulant response. If GlcCer levels do not influence the anticoagulant response in young thrombotic women, it suggests major gender-based differences in the control of the APC anticoagulant response. As the authors note, GlcCer synthase levels are reduced by estradiol and hence may provide a mechanism contributing to the increased risk of thrombosis associated with pregnancy or the use of oral contraceptives. This is a particularly interesting question since thrombosis in females with factor V Leiden (APC resistance due to a mutation in factor V) is increased markedly with the use of oral contraceptives. One might suggest then that there are other undiscovered factors that modulate the protein C anticoagulant pathway since the in vitro anticoagulant response to APC in thrombotic young women is not correlated with GlcCer levels. Understanding the mechanistic basis for gender- and age-related differences in the control of the protein C anticoagulant pathway may open new diagnostic and therapeutic regimens for thrombosis patients.

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Fractalkine and atherosclerosis: a new role for a curious chemokine

Despite the introduction of potent lipid-lowering drugs, coronary artery disease remains the leading cause of death in the Western world. Although hypercholesterolemia plays an undeniably important role in atherogenesis, there is growing evidence that inflammatory macrophages and lymphocytes that accumulate within the lesion contribute substantially to coronary artery disease. The signals that recruit these cells, however, have remained obscure. Now, Moatti and colleagues (page 1925) provide evidence that fractalkine, a novel member of the chemokine family of chemotactic cytokines, may be one such signal.

Chemokines are soluble, secreted proteins that direct the migration of specific subsets of leukocytes. Which leukocytes respond to which chemokines is determined by the set of chemokine receptors expressed on a particular cell. Fractalkine is a unique chemokine: it exists both as a soluble, chemotactic protein and as a membrane-bound, cell-adhesion molecule on endothelial cells. In both cases, its actions are mediated by CX_3CR1, a 7-transmembrane receptor that is expressed on monocytes, T cells, and NK cells.

Moatti and colleagues show that a polymorphism in CX_3CR1 results in fewer copies of the receptor on the cell surface and a reduced incidence of coronary artery disease in humans, independent of other well-established risk factors. These findings suggest that fractalkine plays an important role in monocyte/T-cell recruitment to the vessel wall. In addition, the findings are consistent with recent studies demonstrating that monocyte chemoattractant protein 1, another member of the chemokine family, is critical for fatty-streak formation in murine models of atherosclerosis. Taken together,

these results suggest that impaired recruitment of inflammatory cells does indeed translate into protection from advanced coronary artery disease, and identify chemokines as potentially important new therapeutic targets for the treatment of atherosclerosis. Whether interrupting monocyte/macrophage recruitment late in the course of the disease will be beneficial remains to be determined.

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Defective DNA repair in CML

The transition of chronic myelogenous leukemia (CML) from chronic phase to blast crisis is marked by the accumulation of a variety of genetic abnormalities that underlie the rapidly fatal progression of CML-blast crisis. Although this transition cannot yet be investigated in CML mouse models, it is believed that the antiapoptotic effects exerted by the BCR/ABL oncoprotein may favor the emergence of cell clones carrying additional genetic abnormalities.

In this issue, Deutsch and colleagues (page 2084) provide evidence for a potentially important novel mechanism that may involve defective DNA repair. Stable and conditional BCR/ABL expression in hematopoietic cells was associated with down-regulation of the catalytic subunit of the DNA-dependent protein kinase (DNA-PK_{CS}). Down-regulation of DNA-PK_{CS} was also detected in $CD34^+$ cells from CML patients. Moreover, DNA-PK_{CS} expression was restored by tyrosine kinase and proteasome inhibitors, suggesting that BCR/ABL tyrosine kinase activates a pathway(s) leading to proteasome-dependent degradation of DNA-PK_{CS}. BCR/ABL-expressing cells with undetectable DNA-PK_{CS} levels exhibited a high frequency of chromosomal aberrations upon exposure to ionizing radiation (IR). IR-induced chromosomal aberrations were markedly reduced in cells incubated with a tyrosine kinase inhibitor.

DNA-PK_{CS} deficiency is associated with defective nonhomologous end joining (NHEJ) repair of double-strand breaks. Thus DNA-PK_{CS} knock-out cells exhibit pronounced radiosensitivity, whereas DNA-PK_{CS} knock-out mice show increased incidence of thymic lymphoma and preneoplastic lesions in other tissues. BCR/ABL-expressing cells with undetectable levels of DNA-PK_{CS} were not markedly more radiosensitive of parental cells or BCR/ABL cells with detectable levels of DNA-PK_{CS}. Deutsch and colleagues suggest that the radiosensitivity induced by DNA-PK_{CS} down-regulation might be counterbalanced by enhanced resistance of BCR/ABL-expressing cells to IR-induced apoptosis. Possibly, the concomitant deficiency in NHEJ repair of double-strand breaks and enhanced resistance to apoptosis by IR or radiomimetic drugs favor the accumulation of genetic abnormalities during CML disease progression.

This study raises interesting questions regarding the frequency of DNA-PK_{CS} down-regulation in CML hematopoietic progenitors and the pathway(s) whereby BCR/ABL elicits DNA-PK_{CS} degradation. It also raises the question of whether BCR/ABL affects the expression of DNA-PK regulatory subunits and whether other repair mechanisms are also perturbed in BCR/ABL-expressing cells. Finally, the availability of several mouse lines with various types of DNA-PK deficiency may provide useful models in which to test whether DNA-PK deficiency accelerates BCR/ABL-dependent leukemogenesis.

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Understanding cellular networks in the year 2001

In this issue, Müller-Tidow and colleagues (page 2091) identify the physical and functional interaction of the transcription factor B-myb with cyclin A1/cdk2, a complex that plays a critical role in regulating the cell cycle. B-myb is one of at least 3 members of the myb family of transcription factors, and like c-myb, it plays a role in hematopoietic cell proliferation. Many aspects of the overlapping and distinct functions of these transcription factors remain to be identified, but in this report phosphorylation of B-myb by the cyclin A1/cdk2 complex is shown to enhance the ability of B-myb to activate gene expression, and in particular, the human cyclin A1 gene promoter. Similar but distinct regulatory proteins can exert specific functions within the cell if their activity is variably regulated in response to changes in the cell cycle or in response to external stimuli. The cyclin A1/cdk2 complex is present only during S phase, thus B-myb should maximally activate gene expression at S phase, when the cyclin A/cdk2 complex is most abundant.

Currently, microarray technologies and subtraction libraries are commonly used to identify the target genes of transcription factors, to characterize the cellular response to cytokines, or to differentiate one cell type from another. This study points out at least one of the limitations of these approaches, namely, their inability to detect posttranslational modifications of proteins, which may be very important in understanding cell cycle–dependent events. Cell cycle–dependent regulation of gene expression is perhaps best typified by the regulation of E2F function, a transcription factor essential for DNA synthesis. E2F is activated by the cyclin/cdk dependent phosphorylation of its binding partner, the retinoblastoma gene product (Rb), which releases E2F, allowing it to activate gene expression. Critical post-translational regulation of function is not identified by measuring RNA levels or even the amount of protein present in the cell. Sophisticated and careful approaches will be certainly required to delineate the precise role of B-myb in hematopoietic development.

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