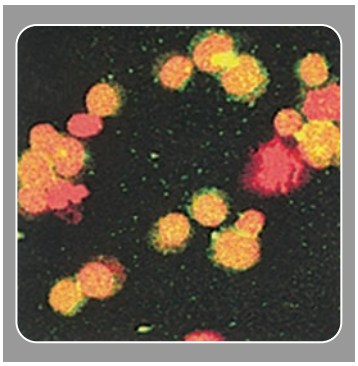
inside blood

REVIEW ARTICLE / HEMATOPOIESIS

Proteomics knocks on hematology's door

The completion of the human genome project and the availability of highly sensitive and accurate mass spectrometers have led to the ongoing proteomics revolution. The proteome represents the population of proteins, whereas the transcriptome describes the population of mRNAs expressed by a cell or tissue. Unlike the genome, the compositions of the proteome and the transcriptome dynamically change with the developmental, hyperplastic, or neoplastic state of the cell. Proteome analysis requires separation of proteins before their identification and characterization. The performance of the analysis depends on the amount of sample protein available and the protein separation technology. Cristea and colleagues (page 3624) in this issue of *Blood* present a review of the proteomics techniques with emphasis on quantitative proteomics.

The capability to monitor differential expression of a gene product, either at the transcript level or protein level, remains at the heart of physiologic genomics/proteomics. Whereas the transcriptome techniques easily lend themselves to relative



quantitation of transcript levels, the proteomics techniques are neither as simple nor as dependable. Also in this issue, Evans and colleagues (page 3751) report on the application of one of the quantitative proteomic techniques for a comparative analysis of hematopoietic stem cell populations. As the authors indicate, their analysis was hampered by the amount of sample protein available, demonstrating one of the still existing fundamental difficulties in applying proteomics to address physiologic questions.

Proteomic and transcriptomic approaches to gene expression analysis each have advantages and disadvantages. Unlike the transcriptomic chip technologies, the proteomic technologies (such as 2-dimensional polyacrylamide gel electrophoresis [2-DE] and isotope-coded affinity tagging [ICAT] in conjunction with mass spectrometry) are not limited to proteins etched on any chip (provided one has a sufficient sample to study) and allow identification of virtually any protein that is detectable (either previously known or unknown). More importantly, the posttranslational modifications (eg, phosphorylation) that may be central to the understanding of gene function are amenable only to investigation by proteomics. Cellular heterogeneity could potentially complicate the interpretation of results generated using either approach. Existing proteomics technologies do not allow analysis at the single-cell level because of the relatively high sample loads required. The advantages of the microarray approach on the other hand are as follows: (1) it is user-friendly; (2) it does not appear to have the problems associated with the core proteomics technology (ie, 2-DE); and (3) most important, as we have recently shown, it facilitates analysis even at the level of single cells.[1] One additional issue is that the correlation between transcriptome and proteome has been known to be poor. The discrepancies are usually explained as due to (1) differences in half-lives of transcripts versus proteins, and (2) differences in the sensitivity of the technology used for detection of the respective gene products. It is also important to recognize that some of these discrepancies may be real, in the sense that they may be intrinsic to the biology of the cell type being investigated. Consequently, such discrepancies could be even greater in stem or progenitor cells in which multiple lineage pathways are simultaneously open at the transcriptional level but not necessarily so at the proteome level.[1] The transcriptome and proteome analyses of a particular cell type may be revealing complementary stories.

Finally, it may help to mention a few cautionary observations relevant to clinical and postdoctoral fellows in hematology. Because proteomic studies are expensive and fairly complex in terms of the numerous sequential technical choices required, it may not be wise to embark upon a proteomics project without the commitment of adequate time, effort, and resources, and a clear working hypothesis. In any event, to assure the uninitiated reader, proteome science is endowed with an excitement of discovery comparable to the Rover landing on Mars. Yet, the pursuit of proteomics is likely to bear fruit in the more immediate future. All systems go!

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1. Seshi B, Kumar S, King D. Multilineage gene expression in human bone marrow stromal cells as evidenced by single-cell microarray analysis. Blood Cells Mol Dis. 2003;31:268-285.

CLINICAL OBSERVATIONS, INTERVENTIONS, AND THERAPEUTIC TRIALS

Bone loss in stem cell transplantation survivors

There is growing use of hematopoietic stem cell transplantation for treatment of primary, refractory, and recurrent malignancies. Although this treatment leads to the salvage of many lives, it is not without potential long-term toxicities. Some toxicities—such as those of the skeletal system—may go undetected until advanced stages are reached, when attempts at amelioration may be unsuccessful and significant compromise of

function occurs. Such is the case with transplantation-induced osteoporosis, which may predispose bone marrow transplantation (BMT) survivors to earlier onset and more severe osteopenia and osteoporosis than the healthy population.

In this issue of *Blood*, Schulte and Beelen (page 3635) present a large prospective study of bone mineral density (BMD) deficits and potential risk factors for its development following allogeneic bone marrow transplantation. This study of 280 adults (median age, 38 years; range, 16-59 years) represents one of the few prospective longitudinal studies of BMD in allogeneic BMT patients. Their extensive statistical analyses of risk factors for rapid bone loss revealed a limited number of factors that directly correlate with BMD loss in this patient cohort: steroid dose, total dose of cyclosporine A, loss of body weight (particularly of muscle mass), and baseline BMD parameters. Interestingly, other potential factors such as age at transplantation, sex, primary diagnosis, pretransplantation regimen, and state of HLA match were not found to be significant factors.

The authors demonstrate that posttransplantation BMD loss is greatest in the first year following transplantation for all sites evaluated. Recovery of bone loss over the subsequent 3 to 4 years was notable and site specific, with the least recovery being seen in the femoral neck and Ward triangle. These site-specific differences suggest an increased risk for proximal femoral fractures in this relatively young patient cohort and should underscore the critical need to develop clinical guidelines directed at optimizing BMD recovery in this patient cohort.

Little information is currently available that addresses effective means of improving BMD in BMT survivors. The authors have provided us with insight into the potential utility of antiresorptive therapy in this patient cohort. They describe a subset of 35 patients in whom antiresorptive therapy was initiated as protection for and/or treatment of osteoporotic fracture after BMT. Of the 10 patients with demonstrated osteoporotic fractures, 9 were younger than 50 years (average age, 31.6 years; median, 39.5 years). Thus, this relatively young cohort seems to be at risk for osteoporotic fracture several decades earlier than the healthy population.

This long-term follow-up study by Schulte and Beelen provides needed details of temporal bone loss related to hematopoietic stem cell transplantation. As they indicate, there is a limited protective effect of younger age at the time of transplantation for spine BMD, the propensity for at least partial restitution of bone loss over time, and an increased risk for transplantation-induced osteoporotic fracture in this cohort. Their work should prompt development of large prospective longitudinal studies to refine risk factors for BMD loss and underscores the need for improved technologic assessments for bone quality, morphology, and quantification in long-term BMT survivors.

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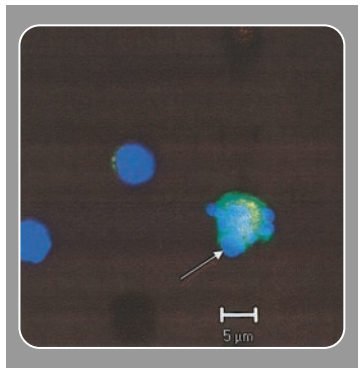
CLINICAL OBSERVATIONS, INTERVENTIONS, AND THERAPEUTIC TRIALS

Endothelial apoptosis: the missing link between atherosclerosis and SLE?

It was proposed more than 25 years ago that atherosclerosis arises as a response of the vascular wall to endothelial injury. Evidence accumulated in the last decade showed that this injury could be due to endothelial apoptosis. Most known risk factors for atherosclerosis induce, while treatments and prophylactic interventions have the potential to decrease, endothelial apoptosis.[1] Many of the findings associated with atherosclerosis, such as endothelial dysfunction and activation, can be caused by endothelial apoptosis, and apoptotic endothelial cells are found on the surface of atherosclerotic plaques.[1-3]

Young and predominantly female patients with systemic lupus erythematosus (SLE) are an unusual group manifesting an extraordinarily strong predisposition for the development of early-onset and accelerated atherosclerosis. In addition to the usual risk factors, SLE itself predisposes to premature atherosclerosis in a manner that does not always correlate with markers of systemic inflammation or measures of disease activity.[4]

What causes premature and accelerated atherosclerosis in patients with SLE? In this issue of *Blood*, Rajagopalan and colleagues



(page 3677) describe increased numbers of apoptotic endothelial cells in the peripheral blood of patients with SLE. They compare patients with SLE to 2 control groups: healthy subjects without presence of usual risk factors for atherosclerosis, and patients with known coronary artery disease (CAD). The markedly higher numbers of apoptotic endothelial cells found in young women with SLE, when compared with the older group of predominantly male patients with CAD, suggest that endothelial apoptosis may be responsible for premature and accelerated atherosclerosis in patients with SLE. The authors fail to detect significant differences in the unequal numbers of circulating apoptotic endothelial cells between the 2 control groups in this study, which could be due to the relatively stable clinical status and the use of endothelial antiapoptotic treatments[1] (statins, aspirin, β-blockers, angiotensin-converting enzyme [ACE] inhibitors) in the CAD group. They do, however, find for the first time in human subjects a significant correlation between numbers of apoptotic endothelial cells and endothelial dysfunction; in a previously described primate model, endothelial dysfunction occurred after endothelial cell loss from the vascular wall and was due to endothelial apoptosis.[2]

The results of the study by Rajagopalan et al suggest that future investigations should focus on specific endothelial pro-apoptotic and antiapoptotic factors that may