macrophage lineage, is enhanced by anti-inflammatory stimuli such as glucocorticoids or interleukin-10 and is suppressed by proinflammatory stimuli such as interferon-γ and tissue necrosis factor-α.[2] Thus, the newly described function of CD163 as a mediator of macrophage-erythroblast adhesion in erythroblastic islands may provide insight into the expansion of erythropoiesis in hemolysis as well as the suppression of erythropoiesis associated with inflammation.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● **TRANSPLANTATION**

Comment on Weissinger et al, page 5511

Clinical proteomics: towards diagnostics and prognostics

Visith Thongboonkerd SIRIRAJ HOSPITAL AT MAHIDOL UNIVERSITY

Clinical proteomics has been applied to define a specific polypeptide profile for acute graft-versus-host disease (aGvHD) after allogeneic hematopoietic stem cell transplantation (allo-HSCT). This profile can then be used for accurate prediction of the occurrence and recurrence of aGvHD.

A biomarker has been recently defined by the Biomarkers Definitions Working Group as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathological processes, or pharmacological responses to a therapeutic intervention."[1, pg91] Biomarkers, therefore, have potential values in monitoring health status. They can be used as a diagnostic tool for disease detection and staging, as well as a tool for disease prognosis and prediction of therapeutic outcome or response to an intervention. Additionally, the ideal biomarkers should also be able to predict recurrence or relapse of the disease after treatment.

After the completion of the Human Genome Project, the post-genomic research has been developing rapidly and has focused largely on translating the genomic information to clinical applications. Clinical proteomics has become one of the most interesting fields with an ultimate goal of biomarker discovery for earlier and more accurate diagnosis, and for more accurate prognosis of the disease. Although the main focuses are diagnostics and biomarker discovery, clinical proteomics also covers identification of new therapeutic targets, drugs, and vaccines for better therapeutic outcomes and successful disease prevention. Recently, a group of proteomists, clinicians,

biochemists, chemists, bioinformaticians, and statisticians from more than 25 institutions worldwide have discussed and begun to define the field and to set adequate standards for clinical proteomics.[2] Through this collaborative effort, clinical proteomics has been defined as "the application of proteomic analysis with the aim of solving a specific clinical problem within the context of a clinical study."[2,pg149] A clinical proteomic study should begin with a well-framed clinical question or problem, followed by selection of the appropriate study populations, samples to be analyzed, and technology to analyze the samples.[2]

Although some single biomarkers have been identified for particular diseases, it has been suggested that a single ideal biomarker may not exist for every disease. Evaluation of a panel of multiple potential disease-specific biomarkers is, therefore, crucially required; proteomics serves as an important tool for such purpose to examine the molecular signature of proteins in a biological sample (proteome profiling). One of the proteomic methodologies that is suitable for proteome profiling is capillary electrophoresis coupled to mass spectrometry (CE-MS).[3] Advantages of CE-MS include automation, high-throughput capability, high sensitivity, and less demand of sample volume.

In this issue of *Blood*, Weissinger and colleagues have applied state-of-the-art proteomic technology using CE-MS to evaluate urinary polypeptide profiles (patterns) of 141 allo-HSCT patients from 5 centers in Germany and the United States. Using this technique for analyzing a training set of 13 urine samples from patients with aGvHD and 50 samples from patients without aGvHD, they defined the
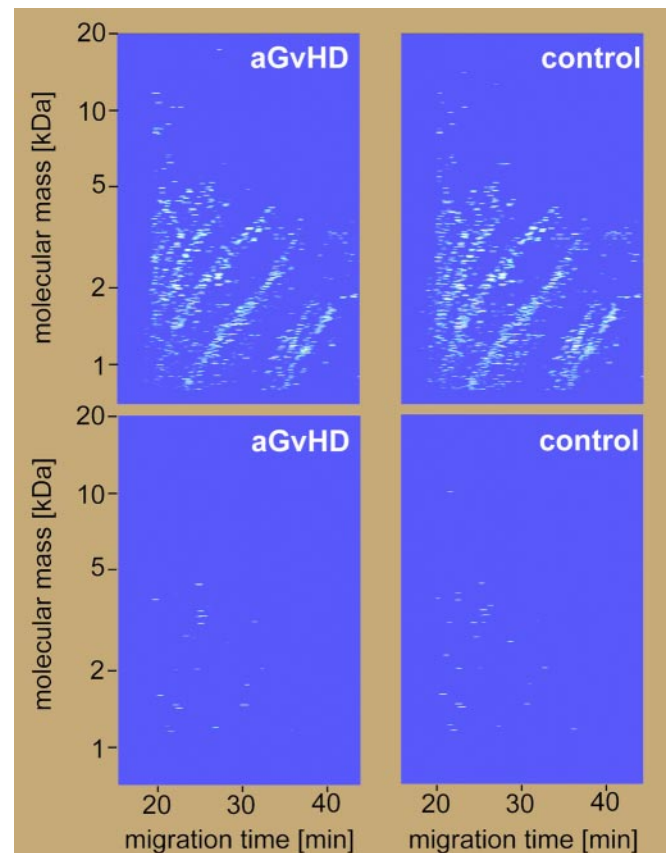
Analysis of urinary polypeptide patterns of aGvHD and non-aGvHD using CE-MS. See the complete figure in the article beginning on page 5511.

aGvHD-specific urinary polypeptide pattern. A model of multiple potential biomarkers containing 31 polypeptides allowed accurate classification of urine samples in the training set with a sensitivity of 100% and specificity of 98%. This panel of multiple potential biomarkers was then used for classifying 599 urine samples in the validation set and provided satisfactory sensitivity and specificity (83.1% and 75.6%, respectively). In addition, urine samples from healthy individuals and patients with other diseases were also analyzed as the quality-control set. Moreover, their findings, particularly the classification factor or support vector machine (SVM) score, could be used for prediction of the occurrence and recurrence of GvHD.

This study is an excellent model to underscore the applicability of clinical proteomics to diagnostics and prognostics. However, a limitation of CE-MS should be also noted. Any proteins with molecular masses greater than 20 kDa are not suitable for CE-MS analysis and, thus, require other complementary methods.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● ● **HEMOSTASIS** ▬▬▬▬▬▬▬▬▬▬▬▬

Comment on Venneri et al, page 5276

Monocytes TIE(2)d up in murky business

George Coukos UNIVERSITY OF PENNSYLVANIA

TIE2-expressing monocytes, a specialized population of tumor-infiltrating monocytes committed to promoting angiogenesis, are now found also in human tumors.

Tumor-infiltrating myeloid cells have been implicated in tumor progression. Monocyte populations with different immunophenotypes have been isolated to date from murine or human tumors by various groups and have been shown to carry out diverse functions, all of which promote tumor growth. The full characterization of these populations is thus expected to provide new opportunities for cancer therapy. In this issue of *Blood*, Venneri and colleagues describe monocytes expressing the angiopoietin receptor TIE2 in human solid tumors. This is an important finding, as their murine counterparts, previously described by the same group, are indeed required for the vascularization and growth of several murine tumor types.[1] Importantly, human TIE2-expressing monocytes (TEMs) represented the main monocyte population isolated from human solid tumors other than canonical tumor-associated macrophages (TAMs). In this report, TEMs markedly promoted angiogenesis in xenotransplanted human tumors, while canonical TAMs depleted of TEMs did not. This complements previous evidence that monocyte populations are paramount for tumor angiogenesis, or sprouting of endothelial cells from existing vessels, and perhaps vasculogenesis, which entails differentiation of recruited endothelial myeloid progenitors into endothelial cells.

In the mouse, Gr-1+ CD11b+ tumor monocytes (or myeloid suppressor cells) promote angiogenesis via paracrine mechanisms and function as vascular-cell precursors.[2] Similarly, VEGFR-1+ CD11b+ monocytes are recruited by vascular endothelial growth factor and exert proangiogenic activity in mouse tumors. Additional monocyte precursors committed to tumor angiogenesis and possibly vasculogenesis include the vascular leukocytes, a subset of CD11c+ MHC-II+ dendritic-cell precursors expressing endothelial vascular markers VE-cadherin, CD34, and CD146. In the mouse, these have been recruited to tumors via CCR6, whereupon they greatly accelerated tumor vascularization and growth.[3] Human vascular leukocytes have been described in high numbers in human ovarian cancer and have been shown to form human neovessels in the mouse, demonstrating vascular commitment.[4] Venneri and colleagues have shown that human TIE2-expressing monocytes also demonstrate clear commitment to tumor angiogenesis, as they can migrate towards angiopoietin-2, a TIE2 ligand released by activated endothelial cells and angiogenic vessels, and that they largely contribute to the tumor angiogenic process in vivo.

The discovery of these tumor-bound monocyte populations offers numerous therapeutic opportunities. First, given the propensity of TEMs as well as vascular leukocytes (VLCs) and possibly other TAMs to home to tumors, and specifically to the tumor vasculature, they can be used as cellular vectors to deliver therapeutic payloads to these targets in a "Trojan horse" cell-based therapy approach. Second, their selective elimination is expected to provide therapeutic benefit. Previous evidence in the mouse has shown that depletion of TEMs through genetic manipulation[5] or of VLCs through immunotoxic methods[4] prevented angiogenesis and induced tumor regression. Identifying specific molecular targets in these populations will therefore be important in achieving selective elimination without toxicity, but could yield important results in the clinic. Finally, understanding the mechanisms that induce the differentiation of myeloid precursors towards these lineages may provide novel ways to re-educate these cells towards a tumoricidal, rather than a cancerophilic, phenotype. Time will show whether one or more of these tumor-infiltrating monocyte populations represent indeed one of tumors' Achilles heels in the human.

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