Although graft-versus-host disease (GVHD) is a life-threatening complica
tion of allogeneic hematopoietic stem cell transplantation (HSCT), its current diag
nosis mainly depends on clinical manifestations and invasive biopsies. Early diagnosis of GVHD, preferably based on unbiased laboratory screening
tools, may increase the safety of allogeneic HSCT and thus further broaden its applica
tion to even larger patient populations. In the past, many efforts were made to use single-protein biomarkers, which were specific for infection or inflammation after allogeneic HSCT but not specific for acute GVHD. Although some of these reports seem to hold promise, in many cases there was a high probability that a single marker was not specific, thus making the diagnostic potential of similar diseases difficult. It is reasonable to believe that the simulta
neous monitoring of more than one protein or peptide within a sample holds greater promise for the differential diagnosis of diseases, including GVHD.

Recently, the application of proteomic tools allowing screening for differentially expressed or excreted proteins in body fluids is becoming more important. Using proteomics, a Japanese group from Sapporo screened for plasma pro
tiens specific for GVHD of grade II or more and 50 control samples from patients with acute GVHD of grade II or more and 50 control samples from humans. In human samples, the serum concentration of CCL8 corre
lated closely with GVHD severity. All non-GVHD samples contained less than 48 pg of CCL8 per mL. In sharp contrast, CCL8 was highly up-regulated in GVHD sera. Strikingly, two patients with severe fatal GVHD had extremely high levels of CCL8. Thus, CCL8 seems to be a promising specific serum marker for the early and accurate diagnosis of GVHD.

This study is of major importance for several reasons:

It confirms preliminary results reported by Weissinger and co-workers’ pub
lished in Blood in which authors describe the application of capillary elec
trophoresis coupled online with mass spectrometry to 10 samples from 10 patients with acute GVHD of grade II or more and 50 control samples from 23 patients without GVHD. About 170 GVHD-specific polypeptides were detected and as a result a tentatively acute GVHD-specific model consisting of 31 polypeptides was chosen, allowing correct classification of 13 of 13 acute GVHD patients and 43 of 50 control samples in a training set. The subse
quent blinded evaluation of 599 samples enabled diagnosis of acute GVHD greater than grade II, even prior to clinical diagnosis, with a sensitivity of 83 percent and a specificity of 76 percent.

The study by Hori and colleagues took advantage of a murine model [in which many parameters could be controlled for] to set up the search for specific markers that allows the characterization of proteins following a huge amount of work that would not have been easily feasible from human samples analyzed by Weissinger and colleagues.1

CCL8 discovery makes the bridge even stronger between chemokines and acute GVHD pathophysiology. Indeed, the migration of cells from vascular to extra-vascular compartments implies a sequential cascade of events, involving interplay between adhesion molecules and chemokines. Acute GVHD requires that effector cells reach their target tissues. Lymphocytes do not enter specific tissues because they “recognize” a given antigen; they enter because they possess the requisite combination of homing receptors and chemokine recep
tors to engage the endothelium at the target tissue[s]. Because GVHD is rela
tively organ-specific — principally affecting the skin, gut, and liver — our increasing knowledge of the pertinent adhesion molecules and chemokines directing effector-cell trafficking to these sites offers novel therapeutic approaches for prevention or treatment of GVHD.

Finally, the use of proteomics opens the door to exciting developments in the
understanding of GVHD, the main one (at least in my opinion) being to use this tool to, finally, try to understand why and how some patients may develop a self-limited disease with an accompanying graft-versus-leukemia effect while others will develop a fatal steroid-resistant disease.


Role of miR223 in Myelopoiensis


New avenues of discovery have opened over the last decade with the discovery of microRNAs (miRNAs). MiRNAs are small RNAs of approximately 22 nucleotide length, which are tran
scribed from genomic DNA like messenger RNAs (mRNAs) but do not encode proteins. Their main function is that of gene regulation by targeting specific sequences in the 3’-untranslated region of mRNAs. It is estimated that the human genome encodes 300 to 500 miRNAs, and that ~30 percent of all genes are regulated by miRNAs. Differential expression of different miRNAs during hematopoiesis was first reported in 2003, and the specific regu
latory functions of several miRNAs have since been elucidated.

The expression and processing of miRNAs has been reviewed in detail elsewhere. Using bio-informatics, the investigators found more than 100 potential target genes for miR223 but focused on mef2c, a tran
scription factor that plays a role in hematopoietic and progenitor cells, and at higher levels in common myeloid progenitors with steadily rising expression with further granulocytic differentiation. In order to investigate the function of miR223, the investigators created mice that lacked expression of miR223 (knockout [KO] mice). These mice showed a surprising finding within the hematopoietic system. Since miR223 expression is upregulated with granulocytic differentiation, it was predicted to promote gran
ulocytopoiesis and hence the mice were expected to lack granulo
cytes. Instead, these mice actually had higher numbers of granulo
cytes, which were hyper-responsive causing a hyperinflammatory state in the mice. The neutrophil count was twice that of wildtype (WT) mice, and this increase was found to be due to an increase in the number of granulocyte progenitors and enhanced neutrophil differentiation.

Using bio-informatics, the investigators found more than 100 potential target genes for miR223 but focused on mef2c, a tran
scription factor known to play a role in myelopoiesis, as it was the only gene with two conserved miR223 complimentary “seed” sites in its 3’UTR (untranslated region). Indeed, when the investigators created mice that lacked both miR223 and mef2c, they found that the mice had normal granulocyte numbers. However, the hyperin
flammatory state persisted. Thus, while the increased neutrophil count of the miR223 mice expressed at least in part through loss of downregulation of mef2c by miR223, a distinct mechanism is likely responsible for the hyperinflammatory state.

The investigators have identified a role for miR223 in regulating granulocytopoiesis and granulocyte activation. MiR223 inhibits translation of Mef2c, a transcription factor that promotes myeloid progenitor proliferation and likely other factors, thereby keeping granulopoiesis “on check”. It is intriguing that the increasing expression of miR223 with granulocytic differentiation appears to function as a built-in repressor or brake in the system, ultimately preventing myelopoiesis, thereby promoting the importance of the regulatory functions of miRNAs in hematopoiesis.


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