inside blood

Sugar in erythropoietin: clinical and forensic implications

Most proteins secreted into the plasma are heavily glycosylated. Complex branched polysaccharides are *N*-linked to certain asparagine residues and *O*-linked to certain serine or threonine residues. The carbohydrate content and structure of plasma proteins are important determinants of their half-lives in the circulation. The cleavage of terminal sialic residues generally results in the rapid clearance of the protein by "asialo" receptors in the liver.

Erythropoietin (EPO) contains 40% carbohydrate, most of which is attached to 3 Asn sites. In the December 15, 2001 issue of *Blood*, Skibeli and colleagues (Blood. 2001;98:3626-3634) report on rather striking differences in the carbohydrate structure of endogenous plasma EPO versus that of recombinant human erythropoietin (rhEPO) produced by high-level expression of the human *EPO* gene in hamster cells. They show that 3 different commercial preparations of rhEPO (alfa, beta, and omega) have a higher molecular weight than endogenous plasma EPO and a high content of fully sialylated tetra-antennary glycans versus none in plasma EPO. The analysis of endogenous EPO was done on protein purified from the plasma of 2 patients with aplastic anemia. The differences in carbohydrate structure between endogenous and rhEPO are likely due to the lack of certain monosaccharide transferases in the hamster rather than modification of rhEPO during circulation in vivo.

These differences in carbohydrate structure are likely to have important clinical consequences. The development of rhEPO is arguably the most successful therapeutic application of recombinant DNA technology. Few pharmaceutical agents can match rhEPO's combination of efficacy and safety. However, there is growing concern about case reports of red cell aplasia developing in patients on chronic rhEPO therapy. The neutralizing antibody that is induced by the recombinant product cross-reacts with endogenous EPO, thereby markedly suppressing erythropoiesis. Because the only structural difference lies in the carbohydrate, it is possible that this is the antigenic stimulus responsible for this rare but serious complication. In this report Skibeli and colleagues demonstrate subtle but significant differences in the carbohydrate structures of rhEPO alfa, beta, and omega. It will be of interest to learn whether these correlate with antigenicity and development of erythroid aplasia. Recently Amgen has developed a superglycosylated rhEPO, Aranesp, that has a markedly prolonged half-life in the circulation and therefore can be administered less frequently. In view of the possible relationship between carbohydrate structure and antigenicity, there will be heightened awareness regarding the development of erythroid aplasia in patients treated with this promising new agent.

In recent years there have been an alarming number of reports of sudden deaths among athletes who have "doped" themselves with rhEPO in order to improve their performance in competition. Prevention of this illegal practice is thwarted by the difficulty in detecting surreptitious use of rhEPO. Radioimmune and ELISA assays in current clinical use cannot distinguish endogenous EPO from rhEPO. Moreover, because the half-life of rhEPO is approximately 5 hours, the levels of plasma EPO will be normal the day following self-administration. The measurement of reticulocytes or surface markers of a cohort of young erythrocytes offers a longer window of time in which the abuse of rhEPO can be detected. Alternatively, the development of a sensitive immunologic assay that can distinguish rhEPO from endogenous EPO would also enable the detection of surreptitious drug use.

—H. Franklin Bunn
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"Special delivery" to cancer cells

Despite 20 years of clinical trials, systemically administered interleukin-2 (IL-2) induces clinical responses in only a very small minority of patients with only a few types of cancer. Based on the assumption that the results are limited largely by inadequate concentrations of IL-2 at the tumor sites and by the toxicity of high dose IL-2, approaches are being explored to deliver IL-2 preferentially or specifically to the tumor.

Carnemolla and colleagues (page 1659) have devised a novel delivery system, namely, a fusion protein between IL-2 and a human antibody (L19) directed against an extracellular matrix (ECM) component of newly forming tumor blood vessels. In this study, the fusion protein (L19–IL-2) administered to tumor-bearing mice selectively delivered IL-2 to the tumor vessels and exerted a significant antitumor effect. The localization to the tumor vessels suggests applicability to "solid" tumors, and even to hematologic malignancies, forming new blood vessels.

IL-2 is not directly cytotoxic and the mechanism for its antitumor effect—whether via T cells, NK cells, or other cytokines—differs for different cancers. Therefore, the clinical therapeutic effect of IL-2 delivered by L19–IL-2 might be restricted to those cancers known to respond to systemic IL-2 or the increased IL-2 concentration at the tumor site might exert an antitumor effect on cancers not known to respond to systemic IL-2. The former possibility would improve the therapeutic index, while the latter would represent a major advance in cancer therapy.

Angiogenesis-associated ECM components represent pan-tumor antigens, which can serve as targets for the delivery of a

inside blood

variety of immunostimulatory or cytotoxic molecules, for example, GM-CSF or IL-12, or toxins or radiation, for example, I^{131} or Y^{90}, selectively to tumor sites for greater antitumor activity and less toxicity. This study represents one such exciting approach.

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Defining the genetic chaos in myeloma

Progress in understanding the malignant transformations that occur in multiple myeloma (MM) plasma cells has been slow, largely due to the significant heterogeneity of genetic and signaling abnormalities, which include extensive chromosomal abnormalities, gene mutations, and deregulated proliferative and apoptotic pathways—indeed, a genetic chaos. Moreover, it remains a major controversy to identify the clonal precursor(s) of the malignant plasma cell. Current prognostic markers have been inadequate to accurately define disease progression, therapeutic response, and clinical outcome. Dr John Shaughnessy and colleagues (page 1745) are first off the block to assemble a high-density microarray of MM plasma cells and to demonstrate that the MM plasma cells are distinctly different than normal plasma cells. In the work presented by Zhan and colleagues, purified plasma cells from 74 newly diagnosed patients and 31 healthy donors were examined for expression of 5 483 genes contained on the Affymetrix HuGeneFL GeneChip. Although the HuGeneFL GeneChip is an early chip design that has been replaced by more extensive and refined gene probe sets, the data presented provide a compelling new definition of MM subgroups and identify a number of new genes as potential therapeutic targets. Hierarchical clustering of plasma cell gene expression demonstrated 4 distinct genetic subgroups. Using links to clinical databases for the patients, the genetic profiles were correlated with clinical outcomes. Many of the genes that distinguish the malignant plasma cell from normal plasma cells were not surprising, though these expectations solidify the validity of the results. The most significant gene expression changes differentiating the MM1 (which included the benign PC dyscrasia, MGUS) and MM4 subgroups code for activities that implicate MM4 as having a more proliferative phenotype and show close similarities with a variety of MM cell lines (validating use of cell line models, at least for the MM4 subgroup). Beyond the clustering of gene expression, Zhan and colleagues identified gene expression spikes in subsets, which reflect the genetic heterogeneity, some of which correlate with genes known to be deregulated by chromosomal translocations. This first written report provides a snapshot of genetic abnormalities that may contribute to the malignant MM phenotype, and additional data presented at the recent ASH meeting have been extended to nearly 150 patients. We certainly anticipate that ongoing efforts to use gene profiling to identify classes of genes may provide a new framework for studying the biology of the disease, mechanisms for its progression, and potential therapeutic targets. And not withstanding the power of gene profiling, protein and signaling profiles will add important components to fully understand and treat this malignancy.

—Brian Van Ness
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