DNA-based diagnosis of red cell enzymopathies: how we threw out the baby with the bathwater

Marinaki and colleagues (page 3327) have identified mutations in patients with pyrimidine-5’ nucleotidase (P-5’N) deficiency at the DNA level. This is a welcome finding, coming as it does years after identification of mutations in all of the more common red cell enzymopathies, such as glucose-6-phosphate dehydrogenase (G6PD) deficiency and pyruvate kinase deficiency, and even of the relatively rare ones, such as triosephosphate isomerase and hexokinase.

Demonstration of mutations at the DNA level is not only of academic interest; it makes possible, as Marinaki and colleagues point out, accurate detection of heterozygotes. It is also useful in diagnosis of patients with the disease: the unborn, women heterozygous for G6PD deficiency, and patients who have received red cell transfusions. In the case of P-5’-N deficiency, it will also facilitate differentiation of patients with inherited P-5’-N deficiency from those who have decreased P-5’-N activity because their red cell population is very old, as in transient erythroblastopenia of childhood or because the enzyme has been inhibited by lead poisoning.

In 1960 we began to recognize that there was great variability in the residual protein in red cell enzyme deficiencies. It was obvious that it would be very useful to be able to find the mutations of patients who had different enzyme variants and different clinical phenotypes. But only very small amounts of enzyme protein were available, and those of us working in this field looked forward to the time that protein sequencing methods could characterize the mutant proteins on a molecular basis.

That is where we threw out the baby with the bathwater.

We purified the enzyme and stored it for that day when technology would have advanced to the point that the protein could be sequenced, and threw out everything else, white cells and their DNA included. No long-term strategic plan had foretold that the decoding of the structure of the protein from the white cell DNA would be the key to understanding mutant red cell enzymes. Now, unfortunately, it will be difficult to apply DNA analysis to patients documented earlier, because usually neither the patient nor the DNA is available. Over the next few years, however, the definition of the structure of the gene and the proof that it is the one involved in the clinical disorder will make it possible to expand our knowledge of this enzyme and the disease that its deficiency causes.

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Parentage and heritage of dendritic cells

Dendritic cells (DCs), despite their common functions of antigen-processing and T-lymphocyte activation, are diverse in surface markers, migratory patterns, and cytokine output. These differences can determine the fate of the T cells they activate. But tracing the ancestry of these specialized DC subtypes has proved to be a dubious enterprise. The original concept that all DCs were of myeloid origin was questioned when Ardavin et al (Nature. 1993;362:761-763) found a mouse thymus T precursor population, which although lacking a capacity to form macrophages or granulocytes, produced thymic DCs. Since these DCs also expressed some lymphoid markers, including CD8α, they were termed “lymphoid DCs.” It was assumed that murine DC lacking CD8α and expressing certain myeloid markers would be the product of myeloid precursors; so these were termed “myeloid DCs.” But our assumption that the early hemopoietic precursor determined the mature DC phenotype was upset when Traver et al (Science. 2000;290:2152-2154) found that bone-marrow myeloid-restricted precursors produced both CD8α- and CD8α+ DCs. Manz et al (page 3333) now complete the picture, demonstrating that lymphoid-restricted precursors are indeed efficient producers of DCs but that both lymphoid-restricted and myeloid-restricted precursors are able to generate both CD8α+ and CD8α- DCs. These results from the Stanford laboratory are in close agreement with our own current findings (Wu and D’Amico, in preparation). Manz et al argue that, since myeloid precursors outnumber lymphoid precursors, most DCs will be of myeloid origin; however, this balance will depend on the relative efficiency of the 2 precursors at generating DCs, on the kinetics of development, and on local environmental influences.

Does this early developmental flexibility mean the ancestry of DCs is irrelevant and that different DC subtypes simply represent different activation states? Not quite. The mature DC subtypes are not readily interconvertible. Recent kinetic evidence (Kamath et al, J Immunol. 2000;165:6762-6770) indicates that the DC subtypes of spleen are the products of separate developmental streams, at least as far back as their immediate dividing precursors. We must now determine the points downstream of the conventional myeloid and lymphoid precursors where the DC sublineages branch off, and determine the cytokines and environmental factors that induce their specialized functions.

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