AMP Deaminase as a Cell-Age Marker in Transient Erythroblastopenia of Childhood and Its Role in the Adenylate Economy of Erythrocytes

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Erythrocytes from 11 patients with presumptive diagnoses of transient erythroblastopenia of childhood were evaluated retrospectively (six) or prospectively (five) for a possible relationship between erythrocyte adenosine 5'-monophosphate aminohydrolase, adenylic acid deaminase (AMP deaminase) activity and intracellular concentrations of adenine nucleotides. Older red blood cell (RBC) cohorts in these patients consistently exhibited significantly decreased activities of AMP deaminase (approximately 5% to 70% of normal control mean) in association with increased concentrations (up to threefold) of adenosine triphosphate (ATP) and total adenine nucleotides. We postulate that the latter is a direct consequence of the former, since diminishing AMP deaminase activity in aging cells should reduce the drain on the adenine nucleotide pool imposed by irreversible deamination of AMP to inosine 5'-monophosphate. Consistent reductions in AMP deaminase activity indicate that this enzyme should also serve as a reliable marker of mean RBC age useful in diagnostic confirmation of transient erythroblastopenia. The observed increases in ATP and total adenine nucleotides in older RBCs require a reevaluation of the traditional view that age-related losses of these compounds mediate the ultimate demise of senescent erythrocytes. Similar alterations in the balance of degradative and salvage pathways in RBC nucleotide metabolism may also underlie certain cases of so-called "high ATP syndrome."

I N V E R Y Y O U N G C H I L D R E N, the idiopathic occurrence of selective erythroid aplasia produces a transient erythroblastopenia of childhood (TEC), a self-limited anemia of progressive severity that generally lasts from several weeks to a few months. Before spontaneous resumption of erythropoiesis, patients with this disorder have a pool of circulating erythrocytes that advances progressively in mean cell age, assuming more of the characteristics of presenescent erythrocytes as the period of aplasia is prolonged. Diagnostic distinction from other causes of anemia may therefore be assisted by quantitative assays of those enzymes that are more active in reticulocytes and young erythrocytes or those that are known to decrease in activity as a function of cell age.1,2

In support of the diagnosis of TEC in one child, we observed diminished activities of a number of these age-dependent enzymes, but also noted virtual absence of adenosine 5'-monophosphate aminohydrolase, adenylic acid deaminase, E.C.3.5.4.6 (AMP deaminase) activity in combination with markedly increased (threefold) concentrations of erythrocyte ATP and total adenine nucleotides. Erythrocytes from both parents had lesser elevations of intracellular nucleotides coupled with intermediate activities of AMP deaminase. This appeared compatible with parental heterozygosity for defects expressed in the child as a homozygous or compound heterozygous deficiency state. The family has not been available for expanded studies to establish or refute unequivocally a hereditary basis for these abnormalities.

Because absent AMP deaminase in the proband might simply reflect enzyme decay in cohorts of older cells populating the peripheral blood of patients with TEC, we reexamined data from previous cases of TEC and prospectively investigated several more. These showed similar, though less severe, enzyme deficits, again associated with significantly increased adenosine triphosphate (ATP) and total adenylates. These observations support those of Suzuki and Dale who reported increases of approximately 75% in ATP concentrations in the oldest fractions of rabbit erythrocytes aged in vivo.3 In a previous report4 and in a companion article,5 Dale and Norenberg also related these ATP elevations to cell age-dependent losses in AMP deaminase activity.

The combined findings of Dale and associates together with the TEC data we present challenge the traditional concept that deterioration of ATP-generating mechanisms in aging erythrocytes ultimately determines their finite life spans. These observations shed additional light on the dynamics of the red blood cell (RBC) adenine nucleotide pool, since deamination of AMP to inosine 5'-monophosphate is an irreversible reaction in mature human erythrocytes. The possibility that some cases of so-called "high ATP syndromes" might involve AMP deaminase alterations should also be considered. This enzyme does appear to be particularly dependent on RBC age and therefore should serve as a sensitive indicator useful in distinguishing TEC from other forms of childhood anemia.

MATERIALS AND METHODS

Blood specimens from all patients were obtained by their primary care physicians under the guidelines of their respective human subject protection committees and referred to our laboratory for investigation of anemias of unknown etiology. The children ranged in age from 5 months to 3 years 6 months, and most had an initial clinical diagnosis of TEC.

Venous blood anticoagulated with heparin was air-expressed under refrigeration to the UCLA Hematology Research Laboratory. Saline suspensions of erythrocytes free of other formed elements were prepared by cellulose filtration and assayed for enzymes involved in aerobic and anaerobic glycolysis, glutathione synthesis. From the Department of Pathology and Laboratory Medicine and the Department of Medicine, School of Medicine, University of California, and the Veterans Administration Center, Los Angeles, CA.

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and cycling, and nucleotide metabolism as prescribed by internationally standardized procedures. Pyrimidine 5'-nucleotidase and deoxyribonucleotidase activities were assayed according to methods reported previously. In some instances, limited availability of blood restricted assays to enzymes known to be reliable markers of mean RBC age useful in distinguishing between TEC and other forms of anemia.

Ultraviolet absorption spectra were determined at neutral and acid pH on extracts of saline-washed erythrocytes prepared by mixing aliquots containing approximately 3 x 10^6 cells/μL with equal volumes of 1.2 N perchloric acid, centrifuging, and diluting the clear supernates with 9 vol 0.1 N hydrochloric acid. In some instances, absorption spectra were also determined on heparinized whole blood specimens similarly extracted with perchloric acid. Spectra were redetermined after treatment of the extracts with barium and zinc salts to remove nucleoside mono-, di-, and triphosphates.

Total adenine nucleotide concentrations were derived from measurements of perchloric acid-extract absorption maxima at 257 to 258 nm using a millimolar extinction coefficient of 14.8. In five cases, adenine nucleotides were quantitatively measured in toto or individually by enzymatic assay of neutralized buffered extracts according to procedures described previously.

### RESULTS

All 11 children were markedly anemic. Pretransfusion hemoglobin concentrations ranged from 1.5 to 6.0 g/dL, averaging 4.2 g/dL, and reticulocytes ranged from <0.5% to undetectable. Standard assays failed to reveal specific defects in erythrocyte enzymes to account for the observed anemia. Other potential etiologies such as hemoglobinopathies or autoimmune reactions were eliminated by appropriate studies before referral of the specimens to our laboratory. Quantitative assays of erythrocyte enzymes observed to have diminished activities in TEC strongly supported that diagnosis in all but one child.

Ultraviolet absorption spectra of erythrocyte extracts uniformly exhibited maxima in the region of 257 to 258 nm, and absorption ratios at different wavelengths were those expected for adenine compounds. No other purines or pyrimidines were detected in the extracts either before or after removal of the nucleoside mono-, di-, and triphosphates by barium hydroxide/zinc sulfate precipitation.

Estimates of total adenine nucleotide concentrations, calculated from absorption spectra maxima, are shown in Fig 1 as a function of AMP deaminase activity. Such measurements do not distinguish among mono-, di-, and triphosphate forms of the nucleotides but reflect the total amount of purine base in all its forms. In all but one instance, nucleotide concentrations were increased more than 3 SD above normal control mean. AMP deaminase activities were all decreased more than 1 SD below control mean, and half were decreased by nearly 3 SD or more. In general, the greatest elevations in adenylate concentrations were associated with the lowest AMP deaminase activities.

In five cases examined prospectively, the increased adenine nucleotide concentrations indicated by the ultraviolet absorption spectra were confirmed by quantitative assays of ATP, adenosine diphosphate, and AMP individually or collectively, or both. These direct enzymatic assays agreed within 5% to 20% with the values derived from the spectral scans, indicating that the latter provide reliable estimates of total intracellular nucleotides in the absence of more sensitive direct measurements. ATP constituted 80%, 84%, 88%, 88%, and 89% of total erythrocyte adenylates in these five cases, and the relative amounts of mono-, di-, and triphosphate forms did not differ substantially from the partitioning usually observed in normal control erythrocytes.

### DISCUSSION

Most studies of structural and functional alterations in aging erythrocytes have relied on in vitro or in vivo cell aging or on fractionation techniques based on buoyant density, osmotic lysis, corpuscular volume, or other characteristics known to be dependent to some degree on RBC age. Transient erythroblastopenia of childhood represents a unique but imperfect model system for such studies. Abrupt cessation of erythropoiesis eliminates influx of new young cells into the dynamic equilibrium of the peripheral blood compartment, while circulating cells continue to mature, age, and die. This results in a cohort of erythrocytes that has a narrower age-range distribution and an older mean cell age as time elapses. In addition, the absence of reticulocytes, with their more extensive metabolic capacities, eliminates many of the disadvantages of in vitro fractionation techniques in which some cross-contamination between young and old cells is unavoidable. Unfortunately, the precise mean cell age of the surviving cohort in TEC remains indeterminant, since the onset of erythropoietic shutdown can be neither anticipated nor detected with assurance or precision. Patients with TEC therefore constitute a heterogeneous study group with variable, as well as uncertain, mean RBC ages.
Nonetheless, as the time interval lengthens after interruption of erythropoiesis, the circulating RBC population ages and progressively assumes more and more of the structural and metabolic characteristics of senescent erythrocytes. When hemoglobin concentration decreases to as low as 1.5 to 1.8 g/dL, for example, as it did in one of these patients, the surviving erythrocytes probably constitute a 10% to 15% fraction of the oldest cells that would normally be in the peripheral circulation. Because such narrow and relatively pure cell fractions are unobtainable by most cell separation techniques, these experiments of nature provide special opportunities to study the aging erythrocyte.

In the index case that prompted this review, threefold elevations in nucleotide concentrations were accompanied by virtual absence of AMP deaminase activity (0 to 0.1 U). None of the other cases we present showed such severe alterations in these two parameters, but all deviated consistently in the same directions, some markedly so. Whether the abnormalities in the initial patient were due to hereditary deficiency of AMP deaminase or to TEC or perhaps to both in combination remains to be determined. Since other cases of hereditary AMP deaminase deficiency have not been associated with anemia, embryolastopenia was probably responsible for the anemia in this patient.

Regardless of the cause of the enzyme deficiency in the index case, the sense of these combined data remains clear: Older RBC cohorts in TEC have increased concentrations of adenine nucleotides in association with diminished AMP deaminase activity. This is contrary to a long and widely held presumption that deterioration in the ATP-generating capacity and diminution of the adenine nucleotide pool itself are principal limiting determinants of RBC life span. However, these findings are entirely consistent with those of several other studies. Of four cases of hereditary absence of AMP deaminase previously reported, ATP concentration was measured in one case and found to be 1.6 times greater than in normal controls. Suzuki and Dale studied biotin-labeled rabbit erythrocytes and reported progressive increases of up to 75% in ATP concentrations in senescent RBCs, and Dale and Norenberg have also related these to decreased AMP deaminase activities. In addition, Dale and Beutler observed isolated examples of either ATP elevation or decreased AMP deaminase in several cases of TEC (personal communication, October 1988).

The association between diminished AMP deaminase and elevated ATP is probably not coincidental, since it has now been observed consistently in three diverse conditions: in fractionated aged rabbit erythrocytes, in hereditary AMP deaminase deficiency without anemia, and in anemic children with TEC. If a causal relation truly exists, its explanation probably lies in the variable changes that occur in certain enzymes as erythrocytes age.

Several reactions influencing intracellular adenylate concentrations are shown in Fig 2. Among potential salvage reactions, adenine is not considered a physiologically important source of nucleotide replenishment, since complete deficiency of adenine phosphoribosyltransferase is unaccompanied by detectable alterations in RBC nucleotide concentrations or life spans. In contrast to adenine, adenosine appears to play a pivotal role in maintenance of the adenine nucleotide pool. Increased or decreased availability of plasma adenosine, resulting from adenosine deaminase deficiency or hyperactivity, is accompanied by expansion or depletion, respectively, of erythrocyte adénynates.

In our experience, adénylate kinase, adenosine kinase, and adenosine deaminase do not change appreciably in activity as RBCs age, whereas nucleotidases and AMP deaminase decrease significantly in activity as reticulocytes mature and erythrocytes progress toward senescence. We showed previously that AMP catabolism preferentially occurs through deamination to inosine 5'-monophosphate rather than by direct dephosphorylation to adenosine. We postulate that this reaction, particularly in younger cells with more active AMP deaminase, imposes a drain on the adenylate pool that is normally compensated by the adenosine kinase salvage reaction. As erythrocytes age and AMP deaminase decays, continued salvage pathway activity could account for progressive expansion of the adenine nucleotide pool in a manner analogous to that observed with adenosine deaminase deficiency.

Regardless of the mechanism involved, these observations demonstrate that progressive loss of ATP cannot confidently be indicted as the ultimate determinant of erythrocyte life-span. Senescent RBCs appear to be removed selectively from the peripheral circulation well before deterioration of their ATP-generating capacity. In this light, age-dependent changes in cell membrane characteristics take on added significance. Alternatively, alterations in metabolic capacities, other than those that maintain ATP and membrane structure, may ultimately emerge as contributory factors.

Although these data indicate that normal elimination of senescent RBCs cannot now be ascribed to lack of ATP, metabolic depletion induced by a variety of conditions clearly can affect erythrocyte viability adversely. This is demonstrable in vitro by the autohemolysıs test of Selwyn and Dacie and by the incubated osmotic fragility test, and is further supported by data derived from studies to determine optimal conditions to extend viability of stored blood. In glycolytic enzymopathies with premature hemolysis, ATP is often...
significantly decreased even in the very youngest erythrocytes (which normally have increased ATP concentrations), and glycolytic intermediates accumulate behind the point of metabolic blockade. Presumably, these abnormalities are exaggerated in any subset of RBCs on the verge of destruction. Similarly, severe reductions in ATP have also been observed with enzyme hyperactivity when the metabolic balance between nucleotide degradative and salvage pathways is unfavorably affected.27

These data also suggest that defective AMP deaminase, (or similar alterations in the balance of nucleotide degradative and salvage pathways), might account for induction of certain “high-ATP syndromes.” Some of these have been explained on the basis of increased pyruvate kinase activity,28 which in and of itself should not increase the total amount of adenylate acids in the cells but might increase the relative amount of ATP. If that occurred, AMP concentration would probably decrease, diminishing its availability for deamination or dephosphorylation and allowing overcompensation by adenosine kinase to expand the total adenine nucleotide pool. In the case recently reported by Staal et al,29 despite twofold increases in ATP concentrations, intracellular AMP was decreased to half that of normal control erythrocytes. Investigation of the possible role of AMP deaminase in some of these cases is in progress.

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