INTRODUCTION

Down syndrome is the most common autosomal chromosomal disorder with incidence ranging from 1 in 700 to 1:1,000 live births [1]. The protean manifestation are mental retardation, congenital heart disease, and risk for early death. The predominant cause of death is related to the congenital heart defects followed by hypothyroidism, respiratory infections, and malignancy (particularly, leukemia) [2,3]. Death from leukemia in Down syndrome children is predominately in the younger ages [2]. Down syndrome children account for approximately 3% of children with acute lymphoblastic leukemia (ALL) and 5–8% of children with acute myeloid leukemia (AML) diagnosed in the United States. Despite the fact that the association of increased risk for leukemia with Down syndrome have now been recognized for nearly 50 years, it is this relatively low frequency of the total number of cases and the reluctance to give aggressive chemotherapy in a developmentally challenged youngster hindered the systematic evaluation of the pathogenesis and treatment of leukemia in children with Down syndrome. Within the last two decades, several important developments in the understanding of the biology and treatment of leukemias in Down syndrome children have occurred. These developments in general define the pivotal role played by chromosome 21, both in childhood ALL and AML. The serendipitous discovery of the unique drug sensitivity of AML in Down syndrome [4] provided additional impetus for these studies. What emerges is a fascinating story for increased risk for leukemia on the one hand and the increased sensitivity to chemotherapy on the other [5]. In this issue of the journal, six articles describe some of these developments and provide new insights on the biology and treatment of leukemia in Down syndrome [3,6–10]. It is a privilege to write this overview. The articles will be reviewed in the context of epidemiology, pathogenesis, and treatment/drug sensitivity. Some of the remaining challenges will be identified in the summation.

Epidemiology of Leukemia in Children With Down Syndrome

Ross et al. [3] describe the current status of the epidemiology of leukemia in Down syndrome. First and foremost, there is a 20-fold increased risk of leukemia in individuals with Down syndrome [11,12]. Brewster and Cannon [13] get credited with the first descriptions of the association between Down syndrome and leukemia in 1930. A striking feature of the increased risk for malignancies is that there is an increased risk for leukemia but not for other solid tumors with the exception of testicular cancer, germ cell tumors, and retinoblastoma [14,15]. The increased risk for leukemia appears to be for the most part to a particularly high risk for one type of AML, megakaryocytic leukemia (AMKL, M7 AML) [16]. The Children’s Oncology Group additionally show an almost fourfold higher incidence of AML to ALL in Down syndrome children [3]. It is fascinating that while the age adjusted incidence of ALL similar to that seen in non-Down syndrome children, the incidence of AML is highly skewed towards the younger age with very few cases if any beyond the age of 5 years confirming the observations from population based registry of Down syndrome children from Nordic countries [14,17].

Comments. Ross et al. [3] offer several suggestions for further investigation of the increased risk for leukemia in Down syndrome. They correctly point out that as of now, the only potentially shared risk factor for both Down syndrome and childhood leukemia is advanced maternal age. Other avenues of study would include quantitation of the exposure to radiation from the diagnostic X-ray studies in Down syndrome for study of heart disease and studies for investigation of intercurrent infections. Ross et al. [3] focus on the relationship of infections in early infancy and the risk for ALL. The infection hypothesis of Greaves et al. [18,19] suggests that exposure to common infections in early childhood may protect a child against ALL by contributing to normal maturation of the immune system, whereas children whose exposure is delayed will be at comparatively higher risk. Greaves et al. suggest that these may be the basis for the lower incidence of childhood ALL in non-industrialized countries versus industrialized.
countries. Children with Down syndrome present a paradox in this regard. On the one hand, the immune deficiency that occurs in Down syndrome may predispose to an increased risk of developing leukemia analogous to the known increased risk of lymphoid malignancies in children with other immune deficiencies. On the other hand, while the age peak for children with Down syndrome and ALL appears to follow the age peak for childhood ALL, the incidence of hyperdiploid ALL and for that matter, ALL with t(12;21) is extremely low or nonexistent in Down syndrome children [8,20,21]. It has been suggested that the increased incidence of childhood ALL in the industrialized countries is largely due to the hyperdiploid ALL. Recent studies from India suggest that the incidence of TEL-AML t(12;21) is lower in the Indian children with ALL [22]. It should also be noted that hyperdiploid ALL is also less common in US black children Smith et al. [23]. Thus, it appears that Down syndrome is protective against the occurrence of hyperdiploid ALL and ALL with t(12;21). This parallels the pattern of ALL in less industrialized (and over crowded, under-nutrition) societies. If so, what may be the basis for this? Could there be a linkage with the relative folate deficiency in Down syndrome and the low incidence of hyperdiploid ALL? An intriguing parallel is the pattern of incidence of leukemia and solid tumors in individuals with homozygosity for C677T variant of methylene tetrahydrofolate reductase, a folate pathway enzyme. Recent studies have shown that homozygosity for C677T MTHFR is protective against hyperdiploid ALL [24] and these individuals are also at a low risk for colon cancer, a cancer not found in Down syndrome children in the population based studies from Denmark [14]. Thus it would, indeed, be great interest to study the folate status of children with Down syndrome, the US blacks (sub-Saharan Africans), and the South Asians (Indian sub continent) patient populations and to correlate with the incidence of hyperdiploid ALL, and ALL with t(12;21) in these populations.

Pathogenesis of Transient Leukemia/AML and GATA1 Mutations


One of the more fascinating manifestations of Down syndrome is the disorder variously known as transient myeloproliferative disorder (TMD or transient leukemia), first described by Schunk and Lehman in 1954 [25]. This disorder is typically seen in newborns associated with a high incidence of spontaneous remissions [26]. The disease is largely clinically silent and frequently discovered by routine blood counts done for other reasons but in some cases the disease is life threatening. In severe cases the infant may be born with hydrops fetalis and show evidence of liver or multi-organ system failure. Retrospective reviews of suggest that neonatal mortality may range from 11 to 55% (the higher figure includes stillborn patients) [27,28]. In the POG 9481 prospective study 8 of 47 patients (17%) experienced early death [29]. And of those who achieve spontaneous remission up to 30% will subsequently develop AMKL [27,28,30]. Megakaryoblastic nature of the neonatal TMD/TL has been clearly established by the electron microscopic studies of Zipursky et al. [31] and as well by the more recent studies using the platelet glycoprotein IIb/IIIa markers CD41/61.

Mutations of exon 2 of the GATA1 gene are universal [32].

Comments. Three important questions remain to be answered. (1) What is the mechanism of the spontaneous resolution of TMD/TL in the majority of the cases? (2) Why do some infants develop hydrops fetalis and liver dysfunction and die? (3) What is the mechanism for the subsequent development of AMKL in up to 30% of the infants with TMD/TL and can this be prevented? All three appear to be linked. The most important new development in the understanding of the biology of AML of Down syndrome is the identification of the truncating mutations involving the hematopoietic transcription factor gene GATA1 (reviewed by Crispino [7]). GATA1 is located on chromosome X and encodes for a zinc finger transcription factor that is essential for normal erythroid and megakaryocytic differentiation. Mutations in exon 2 of GATA1 have been detected almost exclusively in trisomy 21 associated TMD/TL and in Down syndrome patients with AMKL but not in non-Down syndrome AMKL cases [33]. The mutations result in the introduction of a premature stop codon leading to the exclusive production of a smaller GATA1 isoform named GATA1s measuring 40 kDa compared to the normal full length 50 kDa isoform of GATA1. Remarkably these 40 kDa isoform retains both the zinc fingers that are involved in the DNA binding and the interaction site with an essential cofactor named, friend of GATA1 (FOG 1) [7]. It is interesting that all of the GATA1 alterations reported to date abolished the expression of the full length form but retain the expression of GATA1s. This finding is of critical importance with regard to the pathogenesis of AML. For example, mice that totally lack GATA1 (knockout) die in embryogenesis due to deficiencies in both primitive and definitive erythropoiesis, while mice that express low levels of GATA1 (knockdown) with varying degrees of anemia and thrombocytopenia [34]. In these knockdown mice, the GATA1 deficient megakaryocytes are defective in terminal maturation and exhibit abnormal proliferation when expanded in vitro [35]. In parallel to this, it is of interest that blast cells from Down syndrome children with AMKL and TMD/TL express CD36 [36], in contrast to the
low or lack of expression of CD36 in non-Down syndrome AMKL. Further, the expression of wild type GATA1 in the MGS cell line (derived from a Down syndrome child with AMKL) have been shown to partially rescue differentiation [37]. It is to be noted that an earlier study suggested that low GATA1 expression may be a marker for good response in AML [38].

Based on the above and additional observations in aborted Down syndrome fetuses, twins with Down syndrome and prospective studies of screening for GATA1 mutations at birth, Crispino suggests an origin of TMD/TL fetal liver, which would explain both the spontaneous resolution of TMD/TL and the occurrence and the fatal form of TMD and TL with liver failure [7,39]. Crispino speculates that GATA1 mutations occur in fetal liver hematopoietic progenitors in both Down syndrome and unaffected individuals but that the mutations have a selective advantage only against the backdrop of trisomy 21, because of the increased gene dosage effort of key transcription cofactors AML1 and ETS2 which are localized to chromosome 21 [39]. Spontaneous resolution then reflects the transition from fetal liver erythropoiesis to marrow derived postnatal erythropoiesis. The hepatic dysfunction and hydrops fetalis in some of the cases are due to a high TMD burden in the liver resulting in hepatic fibrosis from excessive PDGF and TGF production [6,40]. The later emergence of true AMKL then is due to persistence of these clones or occurrence of the same GATA1 mutation simultaneously in the fetal and marrow derived hematopoietic precursors. This hypothesis supported by the finding of GATA1 mutations in asymptomatic infants with Down syndrome, demonstration of the same GATA1 mutation both at the initial TMD as well as the latter AMKL [41].

The question of whether the children presenting with severe manifestations of TMD/TL should be treated pharmacologically with chemotherapeutic agents remains unanswered at present. Anecdotal data from Toronto shows that low dose cytosine arabinoside (Ara-C) regimen might induce lasting “remissions” in these children and low dose Ara-C regimen has also been shown to be effective in the treatment of myelodysplastic syndrome seen in later infancy in the Down syndrome children [42]. Unpublished data from POG 9481 study also suggests that Down syndrome infants with TMD/TL treated with the low dose Ara-C and who survived have not developed AMKL (G Massey, personal communication). This raises the intriguing possibility that low dose Ara-C,(1–2 courses) may even prevent later occurrence of AMKL. In any case, the treatment of children with hydrops or severe liver dysfunction remains problematic. First, many of these children have far advanced hepatic failure with some autopsy have virtually no viable hepatocytes (unpublished personal observation). In others, there may severe lung disease from either hyperviscosity or pulmonary fibrosis. It is of note that several CCG studies have identified pulmonary toxicity as a feature of the toxicity profile in Down syndrome and leukemia. The unique risk for pulmonary toxicity has not been fully explained although it is of interest that an existing hypothesis for the site of production of platelets suggests that megakaryocytes home in to the lung and then release platelets by explosion [43].

**Treatment of ALL and Down Syndrome**

Basal et al. [8] review the experience of Down syndrome children treated between 1952 study for NIH consensus standard risk ALL (age ≥ 1 and ≤ 10; initial WBC ≤ 50 × 10^9/L). Fifty-nine of 2,174 registered patients or 3% had Down syndrome. The study confirmed the prior observations that hyperdiploid ALL and ALL with TEL/AML1 do not occur in Down syndrome (reviewed by Lange [20]). At the same time, adverse translocations or hypodiploidy was also not observed in the ALL Down syndrome cohort. Prior reviews had suggested that the outcome in Down syndrome children with ALL is either the same or somewhat inferior to ALL in non-Down syndrome, thus contrasting with the markedly superior outcome of AML in Down syndrome versus non-Down syndrome children. In narrowing down into one single study and as well, restricting the analysis to NIH consensus standard risk group, Basal et al. [8] were able to isolate some of the issues. First, among the B lineage ALL patients, as a whole, the ALL Down syndrome cohort had a lower 4 year EFS of compared to the non-Down syndrome ALL cohort but this difference was no longer significant when the groups were adjusted for the presence of either TEL-AML1 or triple trisomies (hyperdiploidy) (78.6% vs. 82.3%, P = 0.14). With regard to overall survival, however, non-Down syndrome cohort did better compared to the Down syndrome group. The authors suggested that the contributing factors for the difference in the low overall survival for Down syndrome ALL cohort might be the increased infection rate or less intensive salvage therapy offered for Down syndrome children with relapse. For example, in their study, only 8% of Down syndrome patients relapsed or received a bone marrow transplant compared to 29% of the non-Down syndrome cohort with relapse. Some other differences emerged in this study. No case of T lineage leukemia was observed in this study in Down syndrome children compared to an incidence of 5.9% in the non-Down syndrome cohort on CCG 1952. The well-known methotrexate related toxicity was confirmed. Down syndrome children spent longer in the hospital than non-Down syndrome cohort, and there was a higher incidence of hyperglycemia. There was also a higher incidence of bacteremia in the Down syndrome cohort. Interestingly, the infection risk was far greater during remission in the
Drug Sensitivity of AML in Down Syndrome

Taub and Ge [10] provide concise review of the series of drug sensitivity studies done by the Wayne State University group, particularly in relation to the unique and endogenous modulation of Ara-C metabolism in Down syndrome. Taub and Ge also provide the initial evidence for the potential linkage of GATA1 mutation and the increased sensitivity to Ara-C. To summarize briefly, the altered folate metabolism in Down syndrome children on account of the increased activity of cystathionineβ synthase (CBS), results in low levels of endogenous dCTP and low s-adenosyl methionine. Low endogenous dCTP results in release of the feedback inhibition deoxycytidine cytidine kinase, the enzyme that phosphorylates both deoxycytidine and Ara-C resulting in a “favorable” Ara-CTP to dCTP ratio. In addition, Taub et al. have shown that expression levels of the Ara-C degrading enzyme, cytidine deaminase (CDA; gene localized to chromosome 1p), are lower in Down syndrome leukemic cells compared to non-Down syndrome cases, an additional factor contributing to the increased Ara-CTP generation in Down syndrome megakaryoblasts [45]. More recent experiments of Taub et al. provide evidence that increased CDA expression could be linked to decreased co-operativity between the mutated GATA1 and a short form promoter of CDA. Stable transfection of the wild type GATA1 coding cDNA into the Down syndrome AMKL cell line, CMK (which contains mutated GATA1 gene), resulted in increased Ara-C resistance and a threefold lower level of Ara-CTP generation. Consistent with this hypothesis are data from pharmacologic interventions aimed at reducing endogenous dCTP by either prior treatment with methotrexate [46] and hydroxyurea [47] or fludarabine [48] (inhibitors of ribonucleotide reductase), which enhance Ara-C cytotoxicity.

**Comments:** While clear evidence exists with regard to this unique modulation of Ara-C sensitivity in Down syndrome, less obvious is the generalized increased sensitivity of Down syndrome AML to anthracyclines and as well, other drugs [44,49,50]. A possible reason appears to be the well-known increased generation of oxygen radicals in Down syndrome observed and the well-documented increased spontaneous apoptosis in multiple cell systems including neuronal cells (reviewed by Ravindranath [5] and Taub and Ge [10] in this issue). A modest increase in superoxide dismutase as occurs in Down syndrome in the absence of a concomitant increase in glutathione peroxidase and catalase might result in increased hydroxyl radical formation which itself might be quite toxic. Studies from the Wayne State group provide additional insight to the increased production of reactive oxygen species (ROS) in Down syndrome cells. Chien et al. [51] demonstrate that an increased mitochondrial production and leakage of superoxide in the CMK cell line compared to non-Down syndrome AMKL cell lines MEG-O1 (derived from a Philadelphia chromosome positive CML case in blast crisis) and HL60 erythroleukemia cell line (also derived from a Philadelphia chromosome positive CML patient). The increased to superoxide production correlates with the increased drug sensitivity of CMK versus MEG-O1 versus HL60. In these studies there was no significant increase in hydroxyl radical production in the CMK cell line, presumably related to an
The discovery of the unique sensitivity of Down syndrome-AML to chemotherapy, the observation of the linkage of reduced function associated GATA1 mutations with Down syndrome-AMKL/TMD have provided a great impetus to understand the mechanistic basis of the pathogenesis of Down syndrome-AML and as well the sensitivity to chemotherapy. The recent studies have proved critical in determining the relationship of folate pathway to cytarabine sensitivity as well the pivotal role of increased ROS in determining cellular sensitivity to chemotherapeutic agents. Several questions that are specific to Down syndrome and leukemia remain to be elucidated. Nevertheless, it is clear that Down syndrome is a unique paradigm for increased risk for leukemogenesis on the one hand, drug sensitivity on the other. Down syndrome may yet become the prototype disorder defining the integral relationship of life (cell proliferation) and death (spontaneous apoptosis).

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