study of transformation mechanisms of MPDs and CML, a full understanding of why the Wistar phenotype is not common to all colonies is important. In particular, eludication of why they are generally more unwell and show transformation to acute leukemia (accelerated phase and blast crisis) may lead to identification of therapeutic targets for clinical intervention.

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Response

Hematopoietic defects in 12/15-lipoxygenase–deficient mice

Based on the comments and data from Taylor et al,[1] we conclude that the various 12/15-lipoxygenase–deficient (Alox15) mice exhibit more similarities than differences.

The majority of Alox15 mice in our colony and those we purchased from The Jackson Laboratory appeared healthy although they had disruptions in hematopoietic stem cell (HSC) function and alterations in numbers of progenitors.[2,3] We initially referred to these as having "chronic" myeloproliferative disease (MPD)[1] and subsequently as "asymptomatic."[2,3] Only 15% of Alox15 mice developed severe MPD and did so at ~ 10-12 months (Figure 1A in Middleton et al[4]), not at 2-3 months as Taylor et al indicated.[1] We initially referred to these mice as "in crisis" because we could adoptively transfer disease to wild-type mice.[4]

Taylor et al imply that the phenotype we reported was unique to our colony,[1] but this is not the case. We obtained similar results with mice from The Jackson Laboratory after housing them in our facility.[2-4] Furthermore, in 2007 The Jackson Laboratory provided confirmation of the phenotype: "We examined spleens from 3 males and 3 females at 12 weeks of age of both mutant and wild-type mice, a similar number of mutant retired breeders and twice as many retired wild-type animals. At both ages, splenic weights of mutants were highly significantly heavier. . . . We also . . . found differences in the proportions of cell types." (Anthony Nicholson, University of Massachusetts, personal communication). Dr Shaoguang Li also studied Alox15 mice at The Jackson Laboratory and has since established a colony at the University of Massachusetts. These mice also have increased splenic weights (2- to 3-fold), disruptions in blood and bone marrow composition, and defective HSC function (A. Nicholson, S. Lin, personal communication, March/April 2012).[2] Therefore, there are in fact no major discrepancies between the "asymptomatic" mice we studied and The Jackson Laboratory and University of Massachusetts colonies.

Alox15 colonies described by Taylor et al share many of these features: (1) mortality rates in Berlin were similar, although it was not indicated whether mortality was associated with MPD; and (2) they report splenomegaly is not apparent at 2 months, consistent with our data showing splenomegaly developed to varying degrees in mice beginning at 8 weeks. Splenic weights in our 10- to 12-week-old asymptomatic mice (up to 0.2-0.3 g) were similar to other colonies (up to 0.29 g) at 7 months (and perhaps earlier as only data for 2 and 7 months were provided).

The use of different methods may explain the apparent disparities with regard to the hematopoietic defects. Furthermore, loss of compartmentalization in spleen was seen only in moribund mice. Alterations in splenic architecture in asymptomatic mice, which we showed were due to increased erythropoiesis,[2] were more subtle. It will be of interest to determine whether splenomegaly in the other colonies is due to similar changes.

A small percentage of our mice developed progressive MPD. Thus, further studies are required to determine whether disease progression is recapitulated in a subset of mice in other colonies

once sufficient numbers of mice in other colonies are observed to 1 year and are subjected to similar analyses. If disease progression is unique to mice in our facility, it might suggest that environmental or infectious agents can play a role in the progression to the more severe phenotype in a subset of mice in the context of 12/15-lipoxygenase deficiency. It is important to stress, however, that the defects we reported in the majority of Alox15 mice that are asymptomatic do not in our opinion appear to be unique to the Wistar colony, and these mice remain a valuable tool for defining the role of 12/15-lipoxygenase in hematopoiesis.

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To the editor:

Dasatinib enhances the expansion of CD56$^+$CD3$^-$ NK cells from cord blood

Dasatinib can inhibit T-cell activation through inhibition of the Scr family of tyrosine kinases such as p56 (Lck).[1] It has been reported that some chronic myeloid leukemia (CML) patients who were treated with dasatinib developed chronic large-granular lymphocytosis (LGL) with natural killer (NK) or NK T-cell lineage, and that these patients achieved optimal molecular response.[2] In addition, Mustjoki et al reported clonal expansion of NK T cells during dasatinib therapy.[3] Kreutzman et al reported that mono/oligoclonal T and NK cells were present in CML patients at diagnosis and expanded during dasatinib therapy and that LGL expansion is linked to cytomegalovirus infection.[4,5] Therefore, dasatinib may have a favorable effect on NK-cell proliferation. In this study, we analyzed the effects of dasatinib on the expansion of NK cells from cord blood and transcriptional factors during expansion.

Umbilical cord blood cells (1×10^6/mL; Hokkaido Cord Blood Bank) were cultured with IL-15 (10 ng/mL; PeproTech), IL-2 (5 ng/mL; R&D Systems), and anti-CD3 mAb (OKT3, 10 ng/mL; Janssen Pharmaceutical); with or without dasatinib (10nM; a kind gift from Bristol-Myers Squibb) in culture medium stem cell growth medium (CeeGenix) with 5% human AB serum in 24-well plates, as we reported previously.[6] After a 7-day culture of umbilical cord blood cells (1×10^6/mL), the absolute number of CD56$^+$CD3$^-$ NK cells had significantly increased in the culture with dasatinib compared with the culture with cytokines only (before culture $5.3 \pm 1.4 \times 10^4$ in 10^6 cord blood cells, after culture with IL-2 + IL-15 $26.0 \pm 17.8 \times 10^4$, and after culture with IL-2 + IL-15 and dasatinib $66.6 \pm 29.1 \times 10^4$; $P < .05$, means ± SDs, n = 6; Figure 1A). In addition, the proportion of CD56$^+$CD3$^-$ cells, CD56$^+$NKG2D$^+$ cells, and CD56$^+$granzyme$^+$ cells significantly increased after culture with dasatinib (Figure 1B).

We analyzed the transcriptional factors Eomesodermin (Eomes) and T-bet using an Applied Biosystems 7300 Real-Time PCR System and GAPDH as an endogenous control. Before stimulation, cord blood of CD56$^+$ cells showed increased expression of Eomes and T-bet compared with the expression in unfractionated whole cord blood cells and CD3$^+$ cells. After 24 hours, Eomes expression was significantly increased in cord blood cells cultured with dasatinib compared with cells cultured with cytokines only (5.96 ± 3.95 vs 0.81 ± 0.62, $P < .05$; Figure 1C).

At present, there are only a few transcription factors that are known to play an essential role in NK-cell development, especially in humans. T-box proteins, T-bet, and Eomes are involved in NK-cell development.[7-9] T-bet and Eomes are both later required for the differentiation in DX5$^+$(CD49b) CD11b$^+$ NK cells. In addition, Eomes is highly expressed in fully differentiated NK cells. In this study, we showed NK-cell expansion after culture with dasatinb and increased expression of Eomes after 24 hours. Therefore, dasatinib has some role in NK-cell expansion from cord blood under the condition of IL-2 and IL-15 stimulation through increased expression of transcription factors such as Eomes. This observation may have potentially important implication for the treatment of other diseases with dasatinib.[10]

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