

mice models of acute lymphoblastic or myeloid leukemia (see figure). Patients had lost almost half of their osteoblasts even before therapy. This is in agreement with a previous report demonstrating functional inhibition of osteoblasts in a murine AML model.<sup>6</sup> In addition, 2 laboratories recently showed that sympathetic neurons are adversely affected by the presence of JAK2-induced MPN or MLL-AF9-induced AML cells, respectively.<sup>7,8</sup> In each case, modifying the number of these microenvironmental cells affected the growth kinetics of the neoplasm. Reducing nestin<sup>+</sup> mesenchymal stem cells (MSCs)<sup>7</sup> or inhibiting adrenergic receptors in sympathetic neurons<sup>8</sup> ( $\beta$ 3 for MPN,  $\beta$ 2 for AML) increased the aggressiveness of the neoplastic disease in vivo. The converse was seen when nestin<sup>+</sup> MSCs were protected through the use of adrenergic agonists, which restored the sympathetic control of these cells. Therefore, it seems that some myeloid neoplasms (and perhaps acute lymphoblastic leukemic cells as well) reduce niche elements that do not support them. The reduction in those niche components appears to be a key contributor to in vivo disease kinetics and a possible cause for the suppression of normal hematopoiesis.<sup>1</sup> Parathyroid hormone injections to mitigate the loss of niche osteolineage cells in studies of BCR/ABL disease<sup>9</sup> or adrenergic agonists in MPN<sup>7</sup> delayed disease progression, respectively. The potency of any of these interventions is unlikely to be sufficient to control disease, but these early studies indicate that a focused effort on niche-targeted therapeutics may be justified. Combining niche support with conventional therapies targeting the neoplastic cell may serve to suppress the malignancy while restoring normal hematopoiesis, thus improving a patient's chances in this duel with disease.

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## ● ● ● PHAGOCYTES, GRANULOCYTES, & MYELOPOIESIS

Comment on Hsieh et al, page 2847

# Resolvin(g) innate immunodeficiencies?

Annalisa Chiocchetti UNIVERSITY OF EASTERN PIEDMONT "A. AVOGADRO"

In this issue of *Blood*, Hsieh et al provided the molecular mechanism showing how X-linked inhibitor of apoptosis protein (XIAP) deficiency may lead to selective innate immunodeficiency recalling X-linked lymphoproliferative syndrome type 2 (XLP-2).<sup>1</sup>

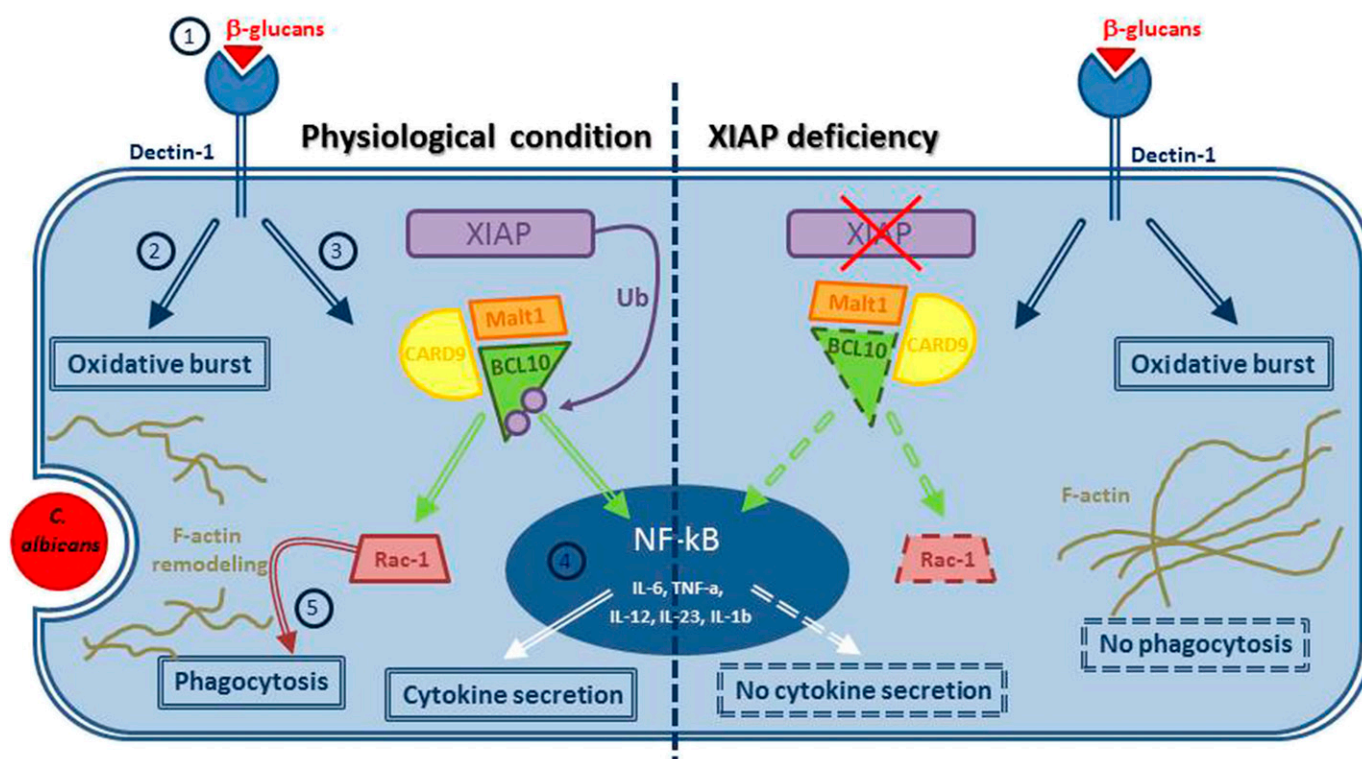
**X**LP-2 is a lymphoproliferative disease associated with XIAP deficiency.<sup>2</sup> Clinical symptoms are mostly attributed to the aberrant activation of macrophages and dendritic cells and the accumulation of activated T lymphocytes, often in response to Epstein-Barr virus infection.<sup>3</sup> This hyperactivation of immune cells leads to hemophagocytic lymphohistiocytosis (HLH), an inflammatory disorder caused by the excess of cytokine production from hyperactivated lymphocytes and macrophages. XLP-2 is also associated with recurrent splenomegaly and fever, but mechanisms underlying the disease are largely misunderstood. Intriguingly, XIAP deficiency may cooperate with other genetic defects of innate immunity to induce the HLH picture.<sup>4</sup>

In this study, the authors show that XIAP-deficient mice are unable to resolve *Candida albicans* infections and confirm this defect in XIAP-deficient human macrophages. Moreover, they show that XIAP is required for the innate responses induced by dectin-1, a receptor belonging to the C-type lectin family and mainly expressed in dendritic cells and macrophages. Dectin-1 recognizes  $\beta$ -glucans that are key cell wall components of several fungi including *C albicans*. On ligand binding, dectin-1 transduces signals through its immunoreceptor tyrosine-based activation

motif in the cytoplasmic domain and activates the caspase recruitment domain family member 9 (CARD9)-BCL10-nuclear factor (NF- $\kappa$ B) axis, resulting in the activation of several genes including those encoding proinflammatory cytokines (see figure). Because of the defective activation of this pathway, *XIAP*<sup>-/-</sup> mice are unable to clear the infection by *C albicans*, whose persistence results in overactivation of macrophages, excessive production of inflammatory cytokines, and splenomegaly. Mice die after 15 days from candida administration. A similar picture is displayed by mice deficient of dectin-1 or CARD-9.

Beside this, Hsieh et al describe a number of important novel observations. They show that XIAP binds and polyubiquitinates BCL10, which is required for the dectin-1-induced innate response and activation of NF- $\kappa$ B. BCL10 contributes to adaptive and innate immunity through the assembly of a signaling complex that plays a key role in the activation of NF- $\kappa$ B triggered through the antigen receptor or FcRs. In XIAP-deficient mice, BCL10 polyubiquitination is missing, and this in turn impaired dectin-1-induced BCL10-mediated activation signals. Interestingly, treatment of *XIAP* mice with curdlan, a dectin-1 ligand, induced an XLP-2 like syndrome, recalling the effects of *C albicans* infection.

## Host defense in fungal infection



In physiological conditions, engagement of dectin-1 by  $\beta$ -glucans composing the fungi wall (1) triggers a complex signaling pathway mediating the oxidative burst (2) and the CARD9/BCL10/Malt1 complex formation (3), which in turn activates NF- $\kappa$ B, with secretion of proinflammatory cytokines (4), and Rac-1, inducing F-actin remodeling and mediating phagocytosis (5). Collectively, these processes lead to the pathogen clearance and consequently to the switching off of the inflammatory burst. In XIAP deficiency, dectin-1-induced innate responses is impaired. BCL10 fails to activate on one side, NF- $\kappa$ B, resulting in no secretion of NF- $\kappa$ B-mediated proinflammatory cytokines, and on the other side, Rac-1, resulting in no phagocytosis. Persistence of *C. albicans* results in overactivation of macrophages, excess production of inflammatory cytokines, and hemophagocytic syndrome. Dashed lines represent the inactive molecules/processes of the pathway.

Moreover, a recent study showed that BCL10 has an NF- $\kappa$ B-independent role in human macrophages, and depletion of BCL10 impairs Rac1 activation, F-actin remodeling, and phagosome formation.<sup>5</sup> Hsieh et al found that dectin-1-induced Rac1 activation is attenuated in mouse and human XIAP-deficient macrophages. Interestingly, they demonstrated that impaired uptake of *C. albicans* by XIAP-deficient macrophages is restored by expression of active Rac1. These results suggest that drugs improving phagocytosis in XIAP macrophages may exert therapeutic effects in XLP2. To this aim, Hsieh et al evaluate the effect of resolvin D1, an anti-inflammatory molecule enhancing macrophage phagocytosis, and show that in vivo administration, 3 days after *C. albicans* infection, fully rescues XIAP-deficient mice from disease and death.<sup>6</sup> Moreover, resolvin D1 restores Rac1 activation and phagocytosis in XIAP-deficient macrophages and results

in effective resolution of inflammation and elimination of *C. albicans*.

Two other interesting points elucidated by this study are as follows. First, these results explain why, when myeloablative conditioning regimens are used in allogeneic hematopoietic cell transplantation in XIAP-mutated patients, a poor survival has been recorded.<sup>7</sup> In fact, XIAP deficiency already impairs the innate reactivity of myeloid cells, and their depletion further aggravates the innate immunodeficiency. Second, involvement of XIAP in BCL10-mediated NF- $\kappa$ B activation may also be relevant for other pathologic conditions such as autoinflammatory syndromes. It has been shown that a common missense polymorphism of the XIAP gene (423Q), associated with recurrent fever, increases XIAP expression, which may influence secretion of tumor necrosis factor  $\alpha$  and interleukin-1 $\beta$  by macrophages.<sup>8</sup> It is intriguing that defective expression of XIAP can cause selective innate immunodeficiency,

whereas its increased expression may favor development of a hyperimmune disease. Therefore, targeting of BCL10 may have therapeutic benefits in different pathological conditions.

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## ● ● ● RED CELLS, IRON, & ERYTHROPOIESIS

Comment on Chakraborty et al, page 2867

# Transfer RNA and syndromic sideroblastic anemia

Achille Iolascon UNIVERSITY OF NAPLES FEDERICO II; CEINGE ADVANCED BIOTECHNOLOGIES

In this issue of *Blood*, Chakraborty et al<sup>1</sup> reported loss-of-function of TRNT1 gene causes a syndromic form of congenital sideroblastic anemia (SA) associated with B-cell immunodeficiency, periodic fevers, and developmental delay (SIFD). This new syndrome, inherited with a recessive pattern, was described in this Journal 1 year ago studying 12 subjects from 10 different families.<sup>2</sup> It is a severe condition with neonatal or infancy onset and systemic tissue involvement that can partially benefit from bone marrow transplantation.

Identification of TRNT1 mutations as SIFD-causing genetic alterations was achieved by using 2 independent technical approaches: genome-wide next-generation sequencing and descendent mapping linkage analysis. Both methods identified germline point mutations of the TRNT1 gene (EC 2.7.7.25) that encodes an essential enzyme that catalyzes the addition of the CCA terminus to the 3' end of transfer RNA (tRNA) precursors. This reaction is a fundamental prerequisite for mature cytosolic and mitochondrial tRNA aminoacylation and quality control as well as for stress response.

Inherited SAs comprise heterogeneous phenotypes depending on the original function(s) of the mutated genes but all characterized by the presence of ringed sideroblasts in the bone marrow aspirate. The latter are erythroblasts with pathological coarse granules of iron deposition in mitochondria, as demonstrated by electron microscopy. Such iron-encrusted mitochondria surround the nucleus of the erythroid cell forming a characteristic “ring,” when stained with Perls' Prussian blue.

Inherited SA is rarer than the acquired form and could be syndromic or not syndromic, with heterogeneous patterns of inheritance. To date,

several genes responsible for inherited SA have been identified and they all play important roles in heme biosynthesis, Fe-S cluster biogenesis, or biology of mitochondria<sup>3</sup> (see figure).

The most frequent form is X-linked SA (XLSA), caused by mutations in the erythroid-specific *d*-aminolevulinic synthase gene (ALAS2). Bergmann et al<sup>4</sup> systematically examined gene mutations in 60 inherited SA probands, and identified mutations of ALAS2, SLC25A38, mitochondrial DNA, and PUS1 in 37% 15%, 2.5%, and 2.5% of cases, respectively. Disease-causing mutations were not found in the remaining 43% of cases, suggesting that there are as-yet-undefined gene mutations that can cause inherited SA.

The observations of Chakraborty et al<sup>1</sup> reduce the number of syndromic forms lacking a causative gene, in particular those with SIFD. Because of the large heterogeneity of SIFD expressivity, it is difficult to calculate the exact percentage of patients carrying TRNT1 mutations because many cases might be misdiagnosed for their mild phenotype. Such cases could now be identified by TRNT1 gene sequencing. The loss of function effect of SIFD-associated TRNT1 mutations was clearly shown in both human fibroblasts and

in yeast, although the specific mechanism by which these mutated proteins cause specifically anemia, immunodeficiency, fever, and developmental delay is still largely unknown. This is another example of an inherited disease in which insufficiency of a single protein, TRNT1, simultaneously promotes the deficiency of many other proteins via the perturbation of protein biosynthesis pathway. Noteworthy is that TRNT1 gene function appears crucial and not redundant for cellular homeostasis and fitness, as demonstrated by the observations that almost all patients retain some enzyme activity and most mutations are hypomorphic. In addition, silencing of the TRNT1 gene is accompanied by cell death in vitro.

However, as in Diamond-Blackfan anemia for the ribosome synthesis<sup>5</sup> and in congenital dyserythropoietic anemia type II for COPII assembly, even if the mutated gene is expressed in a variety of tissues, the clinical symptoms appeared limited to a single or few organs and tissues.<sup>6</sup> How can such an oddness be explained? One possibility could be that, in SIFD, tissues devoted to producing a large amount of a limited set of proteins or even a single one (as hemoglobin for erythroid precursors or immunoglobulin for B lymphocytes) could suffer more from inefficient protein synthesis.

In both the syndromic and not-syndromic forms of SA, the final effect is deregulation of iron metabolism that causes an iron overload in the erythroblast mitochondria associated with increased mitochondrial ferritin, which is, indeed, the hallmark of these anemias. Thus, such iron excess in these organelles needs to be sheltered by mitochondrial ferritin to avoid Fenton-type reactions and iron-induced oxidative damage.<sup>7</sup>

In several nonsyndromic cases (such as those caused by mutations of XLSA, SLC25A38, GLRX5, and FECH), the iron overload appears as a direct consequence of the involvement of a gene whose product is directly related to iron or heme metabolism. In these cases, the symptoms are mainly confined to anemia. On the other hand, syndromic forms appear to be associated with a defect in mitochondrial/cytosolic protein translation (ABC7, SIFD, Pearson syndrome, myopathy lactic acidosis and SA, thiamine-responsive megaloblastic anemia) and in particular with abnormal tRNA function caused by defects in tRNA maturation/modification or even