N-terminal MAML peptides bind Notch and CSL/RBP-Jk but fail to recruit transcriptional activators, thus exerting potent dominant-negative activity (see figure, panel A).[4,9] Dominant-negative MAML (dnMAML) blocks the effects of MAML1-3 family members downstream of all Notch receptors, providing a unique genetic tool to capture the overall impact of canonical Notch signaling.[9] Benveniste et al first built on their past work with OP9 stromal cells expressing Delta-like Notch ligands, now studying highly purified cocultured HSCs (see figure, panel B). Exposure to cytokines and Delta-like1/4 ligands led to Notch-driven progression along the T lineage, but also to expansion of cells maintaining a primitive HSC phenotype and in vivo reconstitution potential. The latter findings were reminiscent of work from Bernstein's group using plate-bound Delta-like ligands.[2,3] Importantly, dnMAML completely blocked both T-cell development and HSC maintenance/expansion, demonstrating that these in vitro effects were mediated by canonical Notch signaling. Next, Benveniste et al studied in vivo functions of Notch signaling by transferring dnMAML-expressing HSCs into irradiated NOD/SCID/γc^{null} neonates (see figure, panel C). This assay was performed in competition with tagged control HSCs, quantifying relative contribution to individual lineages and bone marrow HSCs 10 weeks after reconstitution. In contrast to in vitro findings, dnMAML expression had no impact on the capacity of human HSCs to expand and repopulate xenogeneic recipients, despite complete blockade of T-lineage development (a positive control for efficient Notch inhibition by dnMAML). These observations were reminiscent of findings in mice showing a similar dissociation between in vivo and in vitro effects.[4] Finally, Benveniste et al advance our knowledge of human hematopoiesis by presenting a detailed characterization of cells similar to mouse lymphoid–primed multipotent progenitors.[1]

Thus, observations about canonical Notch signaling translate well from mouse to human HSCs, at least as assessed using HSC-specific pan-Notch inhibition in xenogeneic recipients.[1,4] Benveniste et al relied on the same genetic strategy in vitro and in vivo, providing compelling external and internal controls for efficient Notch inhibition and highlighting the dissociation between in vitro and in vivo effects of the pathway. What might account for these divergent effects? The intensity of Notch signaling delivered to mouse or human HSCs could be lower in vivo than upon in vitro exposure to high-density plate-bound or cell-bound Notch ligands.[2,3] Alternatively, Notch may exert functions on HSCs in vitro that are bypassed by other pathways in the richer in vivo HSC niche environment. As another possibility to account for divergent published observations about Notch and hematopoiesis, Notch may also exert noncanonical effects mediated independently of CSL/RBP-Jk and MAMLs that would not be inhibited by dnMAML expression or CSL/RBP-Jk loss, but still depend on Notch receptor cleavage by γ-secretase. For example, Delta-like1 ligands were suggested to rewire interleukin 6–mediated signal transducer and activator of transcription 3 signaling in $CD34^+$ progenitors through noncanonical mechanisms that remain to be precisely delineated.[10] These effects were identified in bulk $CD34^+$ cells but not in highly purified HSCs, suggesting that they may operate mostly in progenitors downstream of HSCs. Finally, one should highlight that Benveniste et al focused on cell-autonomous effects of canonical Notch signaling in purified HSCs, thus not investigating: other functions of Notch signaling in downstream lineages, a role for Notch at early stages of reconstitution mediated by progenitors downstream of HSCs, possible functions of Notch in serial transplantation or in rare quiescent $CD34^-$ HSCs, or non–cell-autonomous effects that could play a role when Notch is not inhibited only in HSCs.[6-8] Nevertheless, they show an experimental path forward by directly comparing in vitro and in vivo effects of canonical Notch signaling using a modern definition of human HSCs. These careful genetic and functional studies have fundamental significance in the study of human hematopoiesis and practical importance for the development of therapeutic strategies to modulate Notch signaling in patients.

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● ● ● IMMUNOBIOLOGY

Comment on Boggio et al, page 1178

Targeting cytokines in ALPS: it's FAShionable

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In this issue of *Blood*, Boggio et al demonstrate that interleukin-17 (IL-17) can inhibit Fas-induced apoptosis in normal T lymphocytes.[1] More importantly, they

show that blocking IL-17 may be an effective strategy to treat two diseases, autoimmune lymphoproliferative syndrome (ALPS) and Diazani autoimmune lymphoproliferative disease (DALD), which are characterized by defects in the Fas apoptotic pathway.

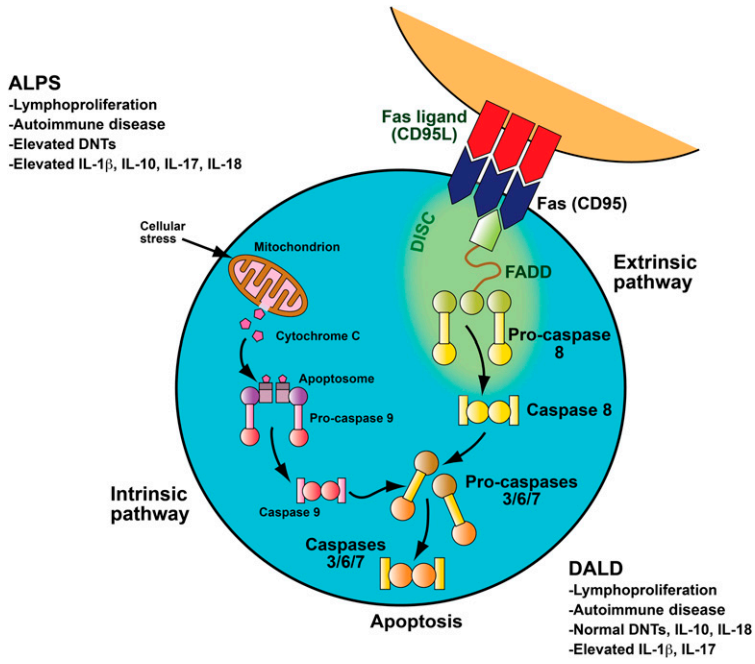
IL-17 is a member of a family of proinflammatory cytokines, including IL-17A and IL-17F, that are primarily produced by a subset of $CD4^+$ T cells known as Th17 cells.[2] IL-17 and Th17 cells have recently been shown to be important in the pathogenesis of a number of autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosis, psoriasis, and Crohn's disease. In addition, they have important roles in cancer immune surveillance and in allograft rejection after organ transplant. Accordingly, a number of drugs that target IL-17 are in clinical development in early-phase clinical trials.

ALPS and DALD are similar disorders characterized by defective Fas-mediated apoptosis, leading to abnormal lymphocyte survival, autoimmune disease, and an increased risk of cancer (see figure).[3] ALPS is diagnosed by a combination of (1) nonmalignant chronic lymphoproliferation; (2) elevated peripheral blood double-negative T cells (DNTs; cell phenotype $CD3^+$, $CD4^-$, $CD8^-$, $TCR\alpha/\beta^+$); and (3) either a genetic defect in an ALPS causative gene (*FAS*, *FASL*, *CASP10*), in vitro evidence of defective Fas function, or abnormal biomarker elevation, such as vitamin B_{12}, IL-10, IL-18, or soluble FAS ligand.[3] Approximately 70% of patients with ALPS develop autoimmune disease, most commonly with autoimmune cytopenias. DALD is a similar disorder that also has chronic lymphoproliferation, autoimmune disease, and defective Fas function. DALD does not have elevated DNTs, but it does have high serum levels of osteopontin.

While many cytokines have been investigated in ALPS and DALD, surprisingly, there is no published work prior to the article by Boggio et al[1] investigating the role of IL-17. Boggio et al make a number of important novel observations. They demonstrate that IL-17 is important in regulating Fas-mediated apoptosis in normal T cells, showing that recombinant IL-17A or IL-17F can block Fas-induced cell death in T cells collected from healthy donors. They also found that IL-17A and IL-17F were abnormally elevated in the sera of ALPS and DALD patients compared with healthy controls. The authors measured additional cytokines previously shown to be elevated in ALPS as controls, including IL-10 and IL-18, finding that both were elevated in their patients. Interestingly, they made the novel observation that IL-10 and IL-18 levels were not elevated in patients with DALD, providing further evidence that ALPS and DALD are distinct diseases. They also found that IL-1β was elevated in both ALPS and DALD patients. This is an important observation because IL-1β is currently targetable in the clinic with anakinra.[4]

Finally, they investigated whether targeting IL-17 could be of therapeutic benefit in ALPS and DALD. They demonstrated that IL-17A neutralization could increase Fas-induced cell death in T cells collected from ALPS and DALD patients. More importantly, they demonstrated that passive immunization with anti–IL-17A antibodies was effective at improving autoimmune disease in a mouse model of ALPS, MRL-*lpr*. Similar to work targeting other cytokines in MRL-*lpr* mice, the effects of IL-17A inhibition were not as robust against the lymphoproliferative manifestations of the disease. Most patients with ALPS and DALD who need treatment require therapy for autoimmune manifestations and not the lymphoproliferative features. Accordingly, IL-17 blockade may be an effective therapy for ALPS and DALD patients, and clinical trials are needed.

Currently, patients with ALPS and DALD are treated with immune suppressive agents, most commonly with corticosteroids, mycophenolate mofetil, or sirolimus.[3] Many patients with ALPS and DALD need life-long treatment, and the use of long-term immune suppression in premalignant conditions could be problematic. In addition, not all patients respond to each available therapy, and some patients cannot tolerate the medicines. Accordingly, new treatment strategies are needed, and cytokine inhibition is a promising potential therapeutic approach.



To downregulate the immune system after systemic insult, activated B and T lymphocytes upregulate Fas, and activated T lymphocytes upregulate Fas ligand. These two interact through the Fas-activating death domain (FADD) to trigger the caspase cascade, leading to cellular apoptosis. Apoptosis mediated through the FAS death receptor is part of the extrinsic apoptotic pathway. In contrast, apoptosis initiated in the mitochondria is part of the intrinsic apoptotic pathway. Patients with ALPS and DALD have a defect in the FAS apoptotic pathway. ALPS and DALD patients develop lymphoproliferation and autoimmune disease. ALPS patients have elevated peripheral blood DNTs, a hallmark of the disease, whereas DALD patients do not. Patients with ALPS typically have elevated serum levels of IL-10 and IL-18. Boggio et al demonstrate that DALD patients do not have elevated IL-10 or IL-18 levels. They also make the novel observations that both ALPS and DALD patients have elevated IL-17A and IL-17F levels as well as elevated IL-1β levels, providing a number of potential therapeutic targets for future investigations. Adapted with permission from a professional illustration by Sue Seif.

Over the past decade, the concept of targeting cytokines to treat autoimmune, rheumatologic, and malignant diseases has evolved at a rapid pace. Currently, there are medicines approved by the US Food and Drug Administration and/or the European Union that target IL-1β, IL-2, IL-5, IL-6, tumor necrosis factor α (TNF-α), interferon gamma (INF-γ), and INF-α.[4] Dozens of medicines that target additional cytokines are in preclinical development and early-phase clinical trials. In some circumstances, these medications are less effective than nonspecific immune suppressants; however, in other situations, they have shown considerable promise. TNF-α inhibitors are very active in rheumatoid arthritis and inflammatory bowel disease.[4] The IL-6 inhibitor tocilizumab may be effective in reversing the cytokine release syndrome seen after novel T-cell activating anticancer therapies, including bispecific T-cell engaging single-chain antibody constructs and chimeric antigen receptor–modified T cells.[5,6] Care is needed and preclinical studies are important before cytokine blockade is used in the clinic, because the cytokine cascade is very complex with positive and negative feedback. For example, IL-10 is markedly elevated in ALPS, leading to the hypothesis that IL-10 inhibition may be effective in ALPS. Yet, in animal models, inhibition of IL-10 caused a worsening of disease.[7]

Further studies are needed to determine the mechanisms underlying the elevated IL-17 levels in ALPS and DALD and to understand the downstream effects of those elevations on different signaling pathways. Signaling through the PI3K/Akt/mTOR pathway can positively regulate Th17 differentiation, and blockade of this pathway by using the mTOR inhibitor sirolimus can downregulate production of proinflammatory cytokines, including IL-17A by Th17 cells in other diseases.[8] Sirolimus is a very active drug in murine and human ALPS, which raises the question: Is part of the benefit of sirolimus in ALPS through inhibition of Th17 and IL-17A? Recent studies have demonstrated that targeting Jak/Stat signaling may be effective in preclinical models of ALPS.[9] Targeting Jak/Stat can impair proinflammatory IL-17 cytokine production, raising another question: Is part of the benefit of Jak/Stat inhibition in ALPS through inhibition of Th17 and IL-17?[10]

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● ● ● LYMPHOID NEOPLASIA

Comment on Xochelli et al, page 1199

Clonal B-cell lymphocytosis: a new member?

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In this issue of *Blood*, Xochelli et al have identified, in otherwise healthy individuals with no clinical signs of lymphoproliferative disorders, a circulating clonal B-cell population with an immunophenotype consistent with a marginal zone B-cell population (CBL-MZ).[1] This scenario is reminiscent of monoclonal B-cell lymphocytosis (MBL)[2] of the "CD5-negative type," although the report includes cases with rather prominent B lymphocytosis (up to 34×10^9/L, K. Stamatopoulos, Institute of Applied Biosciences, Thessaloniki, Greece, written communication, December 16, 2013), being above the MBL threshold of 5×10^9 B lymphocytes/L[2].

It is interesting to note that at the time of the original report, the authors found it difficult to identify cases lacking CD5 expression, while $CD5^+$ cases were more easily classified as chronic lymphocytic leukemia (CLL)-like or non–CLL-like (atypical CLL) MBL.[3] The definition of marginal-type MBL was not widely accepted due to the lack of distinct phenotype for marginal zone (MZ) cells, with the diagnosis being made by exclusion of other more disease-specific phenotypes.

Xochelli et al have demonstrated the occurrence of a circulating monoclonal B-cell population with a distinct MZ-like phenotype using a flow-cytometric profile consistent with a MZ origin. The authors conclusively demonstrate that at least a fraction of the so-called CD5-negative (or expressing very weakly) monoclonal B-cell populations can indeed be defined based on the coexistence of immunophenotypical and morphologic features typically associated with MZ lymphomas involving the spleen (SMZL), including expression of CD49d and FMC7, in the absence of GC markers such as CD10 and/or CD38.

Despite the resemblance to SMZL, some features of CBL-MZ are different. Of particular interest and unexpectedly, none of the reported cases were associated with hepatitis C virus infection, a condition that in the past was considered to underlie most $CD5^-$ MBL cases.[4] Thus, the relationship between the CBL-MZL cases described by Xochelli and