questions regarding Porcn and secretion of Wnts in hematopoiesis but also fuels the catenin-Wnt controversy in hematopoiesis and suggests that catenin-dependent Wnt signaling occurs in the absence of secreted Wnt factors.

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Comment on Sugata et al, page 1095

**A vaccine against HTLV-1 HBZ makes sense**

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In this issue of Blood, Sugata et al report that vaccination against human T-cell leukemia virus type 1 (HTLV-1) basic leucine zipper (bZIP) factor (HBZ) could be used for immunotherapy in adult T-cell leukemia-lymphoma (ATL) patients.1

**The HTLV-1 human oncogenic retrovirus was discovered more than 30 years ago.** It infects 5 to 10 million individuals and is the etiologic agent of both neurologic (eg, HAM/TSP) and hematologic (eg, ATL) diseases. Although a recent report suggests that it is possible to decrease HTLV-1 proviral load in carriers at risk for developing a disease by using a combination of reverse transcriptase and histone deacetylase inhibitors,2 most treatments, when delivered to ATL and HAM/TSP patients, show a modest rate of success.34 The risk of developing ATL is linked to infection early in life. However, transmission could be prevented by screening blood products and avoiding maternal transmission via breast milk. It may be possible to create an anti-HTLV-1 therapeutic vaccine for those who are infected and who develop ATL. Given the extreme genetic stability of HTLV-1, which is linked to the clonal expansion of infected cells rather than to the use of the viral reverse transcriptase,5 it was originally believed that developing an anti-HTLV-1 vaccine would be an easy task.6 The viral envelope is required to bind to the cell receptor(s) and elicits both a humoral and a cellular immune response in infected individuals. In addition, neutralizing antibodies directed toward the viral envelope have been described. Thus, viral envelope-recombinant vaccinia or adenoviruses as well as viral envelope naked DNA and chimeric peptides were generated and used to immunize mice, rats, rabbits, or monkeys in the 1990s and early 2000s.78 However, partial protection was observed in only a limited number of animals after challenge with HTLV-1–infected cells. An animal model in which ATL or HAM/TSP development could be observed at a reasonable frequency was also lacking at that time and did not allow the development of therapeutic vaccine.

Adding another level of complexity, it is well established that HTLV-1 viral expression is extremely low in human ATL patients because of provirus deletion or methylation of the 5' long terminal repeat. This issue needs to be addressed when designing a therapeutic vaccine. The discovery of HBZ, which is encoded by an antisense viral transcript,9 challenged this paradigm. Indeed, HBZ is expressed in infected cells isolated from asymptomatic carriers but, more importantly, in malignant cells from ATL patients. In addition, HBZ transgenic mice develop lymphoma. Thus, this protein is oncogenic and might be considered a target for anti-ATL immunotherapy. Interestingly enough, recent in silico results also suggest that the immune response against HBZ influences proviral load.10

The study by Sugata et al11 aimed to determine whether a recombinant vaccinia virus expressing HBZ as an antigen...
(rVV-HBZ) would elicit specific T-cell responses and whether this vaccine could be used to treat mice inoculated with HBZ lymphoma cells. The HBZ sequence was modified so that the protein could not activate the transforming growth factor β/Smad pathway. First, Sugata et al showed that anti-HBZ cytotoxic T lymphocytes can be obtained in vivo in mice (as well as in rhesus macaques), although HBZ immunogenicity was weak and requires several boosts. Importantly, anti-HBZ CD8+ produces interferon gamma and tumor necrosis factor α. These CD8+ cells recognize and kill mouse T cells pulsed with HBZ peptides and mouse T cells transduced with an HBZ expression vector. More interestingly, CD4+ T cells isolated from HBZ-transgenic animals were efficiently eliminated when transferred to HBZ-vaccinated mice. Thus, rVV-HBZ induces a cytotoxic response against cells that express HBZ in vivo.

More importantly, Sugata et al showed that adoptive transfer of spleenocytes obtained from rVV-HBZ–vaccinated mice significantly improved the survival of animals inoculated with transformed T cells that express only HBZ, as do most ATL cells (see figure). This indicates that rVV-HBZ elicits a T-cell response that is sufficient to eliminate HBZ lymphoma cells in vivo.

Finally, Sugata et al identified an HBZ peptide (amino acids 157–176) that could be used to generate a peptide–based vaccine. Altogether, the results from Sugata et al are important with respect to the generation of an anti–ATL therapeutic vaccine. Now the efficacy of the vaccine needs to be tested against primary human ATL cells.

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Comment on Paggetti et al, page 1106

Exosomes and CAFs: partners in crime

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In this issue of Blood, Paggetti et al present novel findings that chronic lymphocytic leukemia (CLL)-derived exosomes and their molecular cargo are actively transferred to stromal cells that reside in the lymphoid tumor microenvironment (TME), promoting the reprogramming of these cells into cancer-associated fibroblasts (CAFs).1

CLL is characterized by an accumulation of monoclonal CD5+ mature B cells in lymphoid tissues and the peripheral blood. Clonal expansion and invasive migration typically cause the lymph nodes and bone marrow to become infiltrated with tumor. Recent progress in understanding the genetic landscape of lymphoid malignancies must also be coupled with research on the TME. Accumulating evidence is showing that bidirectional communication between cancer cells and their microenvironment is critical for tumor growth, but also profoundly affects therapeutic response. Malignant cells engage in novel associations/interdependencies with stromal cells including fibroblasts (mesenchymal stem cells [MSCs]), endothelial cells, and immune cells that provide crucial contributions to the licensing of tumor progression (survival, proliferation) and immune evasion.2 Many of the examples of heterotypic signaling studied to date involve classical paracrine signaling loops of cytokines or growth factors and their receptors. Although these signaling mechanisms are key mediators of cell–cell communication within the TME, more recently, exosome shedding has emerged as another mode of intercellular signaling. Exosomes are nanometer-sized endocytic vesicles manufactured within multivesicular endosomes and released into the extracellular compartment by many types of cells. Their biogenesis/release is enhanced when cells are stimulated—under stress or in a diseased environment. Transfer of exosomes and their cargo, which includes proteins, messenger RNA, and microRNAs (miRNAs), from cancer cells to other TME cell types has been the subject of intense studies in solid cancers,3 but understudied in lymphoid neoplasia. In this work, Paggetti et al1 provide comprehensive molecular analysis of exosomes derived from CLL cells and, in particular, functional data supporting a novel capacity of these extracellular vesicles to modulate the TME by reprogramming previously healthy stromal cells into CAFs.

The study uses a robust protocol to isolate exosomes from the supernatant of human CLL cells (MEC1 and primary samples). Elegant studies show that tumor-derived exosomes are rapidly taken up by stromal cells, including MSCs, endothelial cells, and myeloid cells (but not CLL cells). Interestingly, this later finding may contrast with microvesicles, a different type of vesicle arising from the outward budding of the plasma membrane, with data suggesting integration into CLL cells.4 Paggetti et al show that active exosome intercellular
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