PIDs with combined T- and B-cell deficiency include defects of DNA repair that carry a well-defined risk of transformation due to genomic instability, not just a lack of effective immune surveillance. Nonetheless, the study by Vajdic et al demonstrates the power of linking data derived from population-based and disease-specific registries to gain insights into the pathophysiology of cancer. With the development of PID registries in many areas of the world, the findings of this study are expected to be confirmed or challenged. Regional differences in the incidence of cancer among patients with PID might also emerge because of the distribution variability of various types of PID, exposure to tumor-promoting pathogens, and frequency of genetic variants conferring cancer susceptibility among different populations.

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Comment on Nakata et al, page 1280

A new window on c-Myb function

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The c-Myb transcription factor is required for adult hematopoiesis. c-Myb is abundantly produced by hematopoietic stem cells and progenitors and is downregulated in terminally differentiated hematopoietic cells. Expression of c-Myb increases in naive T and B lymphocytes after activation but little is understood about c-Myb function in mature lymphocytes. In this issue of Blood, Nakata and colleagues report that c-Myb is crucial for optimal development of human Th2 cells and demonstrate that c-Myb is important to establish autoregulation of GATA-3 expression during Th2 differentiation.

CD4 helper T cells are critical for defense against a variety of pathogens. Depending on the cytokine environment after T-cell receptor–mediated activation, CD4 T cells differentiate into several types of helper T (Th) cells including Th1, Th2, and Th17 helper cells that are defined based on the cytokines that they produce. Interleukin-4 (IL-4)–mediated activation of signal transducer and activator of transcription 6 (STAT6) in activated CD4 T cells leads to expression of GATA-3, which is a transcription factor that is critical for differentiation of Th2 cells. The ability of exogenous GATA-3 to induce Th2 differentiation in the absence of IL-4 or STAT6 suggested that GATA-3 is able to mediate an autoregulation loop during Th2 differentiation. c-Myb has been reported to directly regulate transcription at the GATA-1 locus in mouse thymocytes and to be important for CD4/CD8 lineage decisions. Nakata et al used short hairpin RNA (shRNA)–mediated silencing to identify potential c-Myb target genes in human effector/memory CD4+CD45RO+ CD4 T cells and determined that mRNAs encoding GATA-3 and Th2 cytokines were decreased in the absence of c-Myb. Subsequent silencing of c-Myb expression in CD4 naive (CD45RO+) and effector/memory T cells cultured under Th1- or Th2-promoting conditions led to decreased GATA-3 and Th2 cytokine production while expression of T-bet and interferon-γ was spared. Thus, c-Myb appears to be important for the differentiation and maintenance of CD4+ T cells with a Th2 phenotype. GATA-3 mRNA is transcribed from 2 alternative promoters, exon 1a and exon 1b. The exon 1a promoter is directly regulated by Notch signaling and is crucial for GATA-3 expression in mice during Th2 differentiation. Nakata et al determine that GATA-3 mRNA in activated naive and effector/memory human CD4 T cells parallels increased c-Myb expression during growth under Th2-promoting conditions and, perhaps surprisingly, is almost entirely transcribed from the exon 1b promoter. Whether this difference in promoter use reflects a difference between the human and mouse systems requires clarification. Nakata and colleagues demonstrate that a conserved Myb-binding site in the exon 1b promoter is crucial for activation of exon 1b promoter/reporter constructs in primary human CD4 T cells. Silencing of endogenous c-Myb expression abrogated transcription from exon 1b promoter/reporter constructs in primary human CD4 T cells. Silencing of endogenous c-Myb expression abrogated transcription from exon 1b promoter/reporter constructs, and chromatin immunoprecipitation (ChIP) assays identified c-Myb bound to the endogenous exon 1b promoter in naive CD4 T cells grown under Th2-promoting, but not Th1-promoting, conditions. c-Myb was not found to be associated with exon 1a promoter chromatin. Thus, the GATA-3 exon 1b promoter appears to be a direct c-Myb target in human Th2 cells. Nakata et al further establish that GATA-3 also interacts with the exon 1b promoter near the c-Myb–binding site. GATA-3 alone has little ability to transactivate the exon 1b promoter but c-Myb and GATA-3 cooperate on the exon 1b promoter, suggesting that GATA-3 could establish an autoregulatory loop in the presence of c-Myb during the differentiation of Th2 cells. Few bona fide c-Myb target promoters have been established and little is actually understood about how c-Myb functions as a transcription activator. Nakata et al determined that GATA-3 does not bind to the exon 1b.
promoter in the absence of c-Myb, providing a framework for understanding how GATA-3 can self-regulate transcription in developing Th2 cells (see figure). Interaction of IL-4 with the IL-4 receptor activates STAT6 and allows a small amount of GATA-3 protein to be produced; this alone is not enough to establish an autoregulatory loop. However, in the presence of c-Myb, produced in response to T-cell activation, GATA-3 is able to bind the exon 1b promoter, resulting in the expression of more GATA-3 and potentially continued expression even in the absence of further IL-4 signaling. Nakata and colleagues pursue the nature of the cooperation between c-Myb and GATA-3 and find that the 2 proteins exist in a complex in Th2 T cells that can be immunoprecipitated; all 3 are found to be bound to the exon 1b promoter in Th2 T cells.

Nakata and colleagues previously reported that Menin is able to establish a complex that includes c-Myb, Menin, and mixed-lineage leukemia (MLL). MLL methylates lysine 4 on histone H3 and is crucial for the maintenance of memory Th2 responses in mice. Using a ChIP/reChIP approach showed that MLL joins c-Myb, GATA-3, and Menin on the exon 1b promoter in effector/memory Th2 cells, suggesting that this complex may lead to or maintain changes in the chromatin architecture at the GATA3 locus as the Th2 response matures and CD4 T cells differentiate into effector/memory Th2 cells. Nakata et al indeed identify portions of the GATA3 locus in Jurkat cells as well as differentiating and effector/memory Th2 cells that include the exon 1b promoter and are marked by dimethylation and trimethylation of histone H3 on lysine 4 as well as histone H3 lysine 9 acetylation. Silencing of c-Myb expression by shRNA resulted in decreased H3K4 dimethylation and trimethylation and H3K9 acetylation in both differentiating and effector/memory Th2 cells. Nakata and colleagues speculate that recruitment of MLL to the GATA3 locus may be in part responsible for changes in histone modifications that occur during transition to the Th2 effector/memory stage. However, the notion is not tested. Nakata et al have opened an important new window on c-Myb function but questions remain. For example, it is unclear whether the chromatin modifications identified at the GATA3 locus are dependent on c-Myb action at the exon 1b promoter or are secondary to function elsewhere. There is little difference between the histone modifications detected in differentiating compared with effector/memory Th2 cells yet MLL does not appear to join the c-Myb/Menin/GATA3 complex until the effector/memory Th2 stage. In addition, understanding the relative roles of c-Myb and Notch signaling in the control of GATA3 expression during Th2 differentiation in mouse and human systems will be important. Gaining insight into c-Myb function has been difficult and the article by Nakata et al provides significant new information.

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