IFN-γ and that macrophages contained IL-6 and TNF-α in situ. These findings are notable for 2 reasons. First, they confirm the idea that despite a variety of clinical and etiologic features, patients with hemophagocytic syndromes have similar disease pathophysiology. Second, this study directly demonstrates which cell type is producing each cytokine.

While the observations of Billiau et al do not establish causality, they are consistent with the findings of a murine model of hemophagocytic lymphohistiocytosis, which clearly demonstrated that IFN-γ was essential for disease development.4 The findings of this model and the current paper by Billiau and colleagues are consistent with the theory that hemophagocytic syndromes develop when CD8⁺ T cells secrete excessive amounts of IFN-γ and thereby drive macrophages to toxic levels of activation. How the T-cell response spirals out of control and precisely how IFN-γ is linked to the clinical phenotype of hemophagocytic syndromes are not yet known. Future investigators will need to establish a clear, causal sequence of events that leads to the unfortunate and fatal immune activation seen in the hemophagocytic syndromes.

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**Comment on La Motte-Mohs et al, page 1431, and Lehar et al, page 1440**

**Cooking up T cells**

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The Notch ligands Delta-1 and Jagged-1 differentially induce T-cell development in culture.

Two articles in this issue address how Notch ligands induce T-cell development. Mammalian Notch ligands comprise 2 families, serrate-like (Jagged1, Jagged2) and Delta-like (Dll1, 3, and 4). Both families encode transmembrane proteins whose extracellular domain contains epidermal growth factor (EGF)–like repeats and an N-terminal DSL domain (for Delta, Serrate, Lag2) that binds Notch receptors and short, poorly conserved intracellular domains that have important but poorly understood functions. Notch receptor binding by ligand initiates a series of proteolytic cleavages in the receptor, causing release of the intracellular domain of Notch from the plasma membrane and translocation into the nucleus, where it creates a short-lived complex that activates transcription.

Specificity of Notch ligand–receptor interactions is poorly understood. In some assays, such as inhibition of myocyte development, Delta and Jagged function equivalently. In vivo, Delta and Jagged often show overlapping expression, providing limited insight into specificity. Knockout studies demonstrate that Notch ligands are not equivalent, at least for marginal zone B-cell development. These results show that Dll1 is the important partner for Notch2 in marginal zone B-cell development, as conditional deletion of either leads to loss of these B cells.1

The precise Notch ligands in T-cell development are less certain. Notch1 signals are uniquely required among the 4 receptors for both T-cell commitment from a multipotent progenitor and proper development to the double-positive (DP) stage.2 Conditional deletion of either Jagged1 or Dll1 leaves T-cell development unaffected, suggesting that neither uniquely signals T-cell development and that conditional deletion of multiple family members will be necessary.1,3 A major breakthrough in identifying Notch ligands relevant to T-cell lymphopoiesis was provided by establishing stromal cell cultures that recapitulate many aspects of T-cell development.3 Using either OP9 or S17 stromal cells engineered to express Dll1, Schnitt and Zuniga-Pflucker and Jaleco et al succeeded in establishing a cell culture–based assay that recapitulates T-cell development, a process previously thought to require intact thymic organ cultures. In the absence of Delta–induced Notch signals, these cultures generate B cells. OP9-Dll1 cells are particularly useful as they efficiently generate DP and even mature single-positive CD8 cells from murine hematopoietic progenitors derived from embryonic stem cells, fetal liver, and adult bone marrow. In addition, these cultures have provided important mechanistic insights into murine T-cell development. Jaleco et al also showed that CD34⁺ human cord blood cells were capable, albeit inefficiently, of generating DP T cells on S17-Dll1 cells. In this issue, La Motte-Mohs and colleagues extend the previous studies by showing that culture of human cord blood on OP9-Dll1 cells leads to efficient T-cell commitment, expansion, and generation of T-cell receptor αβ (TCRαβ)–expressing cells. Whether the latter are functionally mature remains to be determined. Similar results were obtained using human adult bone marrow progenitors.4 Thus, it should now be possible to obtain a detailed understanding of early human T-cell development and use this information to manipulate and expand T-cell progenitors for therapeutic purposes.

The ability of OP9-Dll1 cells to induce T-cell development raises the question of whether Jagged ligands have the equivalent ability. In this issue, Lehar and colleagues engineered OP9 cells to express increased levels of Jagged1 (OP9-Jag1). As Jagged1, unlike Dll1, is expressed on parental OP9 cells, this appears to be a quantitative rather than qualitative change. In contrast to Dll1, OP9-Jag1 cells failed to induce T-cell development and induced only weak expansion of committed T-cell progenitors. Nevertheless, Jagged had some effects, as thymic DN1 cells, a heterogeneous population, were unable to form B cells when cultured on OP9-Jag1, as opposed to OP9 control cells. This study and previous work from Jaleco et al demonstrate that Jagged and Delta signals are not equivalent in the context of T-cell development. Whether these differences are quantitative or qualitative is unknown. For example, Fringe-induced Notch modification could lead to Jagged insensitivity. Alternatively, ligand density may play a role, a parameter that has not yet been assessed.

Although much remains to be learned about the mechanism of Notch–induced T-cell development, the ability to efficiently generate human T cells in culture holds great therapeutic promise, such as in improving T-cell generation after myeloablative therapy. The culture systems described to date are an excellent start and understanding the functions of the ligands in producing specific signals will be important. This hope must be tempered with caution, as it is not yet known whether the T cells derived from
these cultures will have an increased propensity to cause autoimmune disease or leukemia. Nevertheless, the advent of these culture systems should allow rapid progress in addressing these issues and translating these findings to the clinic.

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Comment on Yu et al, page 1734

Tissue factor and tumor cells: as bad as it gets?

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Yu and colleagues have characterized sequential transforming events that drive the up-regulation of tissue factor on colorectal tumor cells. Furthermore, tissue factor expression defines the aggressive character of these cells in vivo.

In the last decade, tissue factor has been found to impact nonhemostatic physiologic processes in a dramatic way. The primary initiator of the coagulation cascade is indispensable for normal embryonic development and plays a significant role in inflammatory processes. However, it has also become clear that tissue factor expression and oncogenic processes are tightly associated. Tumor metastasis, for example, appears to be critically dependent on tissue factor overexpression; metastatic cells may express up to 1000-fold more tissue factor than their nonmalignant counterparts. In addition, a number of studies demonstrate that tissue factor is both a significant risk factor for hepatic metastasis in patients suffering from colon cancer and essential for metastasis in a murine pulmonary metastasis model.

Tissue factor has also been found up-regulated on primary tumor cells, and many tumors show a dramatically increased procoagulant activity. Surprisingly, very little is known about the molecular mechanism underlying tissue factor overexpression in cancer cells and the impact of tissue factor overexpression on primary tumor growth. In this issue, Yu and colleagues unravel the nature of the transformation events that lead to deregulated tissue factor production in colorectal cancer cells. They show that K-ras and p53 mutation lead to a cumulative overexpression of both cell surface–exposed tissue factor and circulating tissue factor in the plasma of tumor–bearing mice. Expression of tissue factor was found to be required for the full manifestation of both tumor angiogenesis and aggressive tumor cell behavior in vivo, possibly via regulation of the antiangiogenic factors thrombospondin-1 and 2.

Interestingly, tissue factor expression is not a transforming event per se; tissue factor–associated aggressiveness was found to be strictly dependent on the host environment, since tissue factor dramatically influenced tumor growth properties in vivo but did not alter growth properties in vitro. Therefore, although highly speculative, this finding may point toward a role for tissue factor ligands, such as factor VII/VIIa or plasminogen, in oncogenic events. Since both hemostatic factors have been shown to induce tissue factor–dependent intracellular signaling cascades, they may be the direct trigger of tissue factor–associated tumor cell aggressiveness.

Alternatively, activation of downstream coagulation factors such as FX or prothrombin may facilitate this process.

Understanding the role of tissue factor in primary tumor growth may be of vital importance for the development of new anticancer therapies. Directly targeting the transforming oncogenes is often not feasible, whereas blockade of downstream events, such as tissue factor function, may be more effective. Thus, the work described by Yu et al provides valuable information that may facilitate the use of tissue factor–blocking strategies in cancer.

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Effect of TF gene silencing on HCT116 tumor growth and angiogenesis. See the complete figure in the article beginning on page 1734.
Cooking up T cells

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