

(IgG) anti-HEL. Surprisingly, the transfused RBCs were not hemolyzed; rather, HEL was specifically removed from the transfused RBCs, which then survived normally. This Fc receptor-mediated process did not require the spleen and probably occurred in the liver. Given that extravascular hemolysis, and not antigen suppression, is found in both a mouse model of AIHA<sup>4</sup> and following transfusion of glycoprotein A transgenic mouse RBCs into alloimmunized recipients (David A. Schirmer, Shuh-Chung Song, and S.L.S., unpublished observations, May 2005), it will be interesting to compare and contrast these models in future studies.

Finally, the model of Zimring et al is reminiscent of the “transfer reaction” involving immune complexes bound to complement receptor 1 (ie, CR1) on primate RBCs, in which macrophages ingest both the immune complex and CR1 without producing hemolysis.<sup>5</sup> In particular, the Fc region of the relevant antibody and intact Fc receptor function are required for the transfer reaction.<sup>5,6</sup> In addition, the liver primarily removes the CR1-bound immune complexes in vivo.<sup>6</sup> Although mouse RBCs do not express CR1, this process may not be limited to CR1,<sup>7</sup> thereby suggesting addi-

tional ways of studying antigen suppression in vivo using the mouse model of Zimring et al and in vitro using antibody and RBC samples from human patients. ■

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## ● ● ● TRANSPLANTATION

Comment on Beilhack et al, page 1113

# GVHD: in vivo veritas

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A new imaging technique reveals T cells' journey into GVHD target organs.

**G**raft-versus-host disease (GVHD) remains the major toxicity of allogeneic bone marrow transplantation (BMT) and prevents the broader use of this potentially curative therapy.<sup>1</sup> The liver, skin and gastrointestinal (GI) tract are primarily affected by acute GVHD, although the lung is also probably a target.<sup>2</sup> This distribution of organs is unusual, because kidney and heart allografts are also subject to immunologic attack (ie, rejection), yet they are not targets of acute GVHD. The elegant study of Beilhack and colleagues from Stanford in this issue of *Blood* takes us one step closer to understanding this unusual clustering.

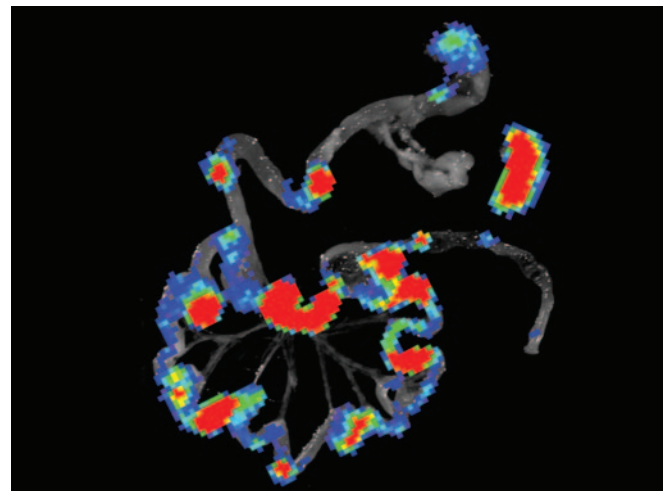
To track the movements of cells in vivo, the Stanford team used a technique of bioluminescence imaging, similar to PET scanning. They first engineered the T cells to glow in the dark, and then tracked their movements in a serial fashion after allogeneic BMT. Within 12 hours, the T cells homed to secondary lymphoid organs

in the abdomen. Within 96 hours, donor T cells had divided at least half a dozen times (a 64-fold increase) and could be easily detected throughout the GI tract (see figure). No such proliferation occurred in recipients of syngeneic BMT that did not develop GVHD. Additional experiments documented increased expression of gut homing receptors on proliferating T cells. In the absence of homing receptors, T cells did not cause GVHD when

transplanted, even though they proliferated in vitro to antigen presenting cells of the host.

These findings provide a fascinating glimpse of early T-cell behavior during GVHD, and they underscore several important points. First, the gut-associated lymphoid tissue and the spleen are the first destinations for T cells in an allogeneic host, and they find their targets rapidly. Other groups have also shown this early migration to the GI tract during GVHD, which helps explain the primacy of the GI tract as a GVHD target organ.<sup>3,4</sup> Indeed, the mucosal surface of the GI tract is largely destroyed within 1 week after transplantation in many mouse BMT models, undoubtedly contributing to the rapid mortality. GVHD prophylaxis strategies that specifically target the GI tract may therefore have broad systemic effects and help reduce transplantation-related mortality.

Second, the speed of T-cell activation and proliferation after infusion reminds us that the T cells causing GVHD are already mature and do not need to differentiate from stem cells present in the donor inoculum. Within hours, T cells migrate out of the bloodstream into intestinal and splenic lymphoid tissue, where they proliferate. This rapid transit time is particularly pertinent in clinical medicine where therapeutic levels of calcineurin inhibitors are often not achieved until several days after stem cell infusion. Subtherapeutic levels at a time of such intense T-cell stimulation may have dramatic consequences on eventual GVHD severity, even though the clinical symptoms may not be apparent for another 2 weeks.



**Bioluminescence imaging (BLI) of gastrointestinal tissues and spleen combined with histology and immunofluorescence microscopy of Peyer patches during induction of acute GVHD. See the complete figure in the article beginning on page 1113.**

Third, the immediate homing of T cells to lymphoid organs reinforces the fact that the primary target organ of the GVHD is the lymphohematopoietic stem of the recipient.<sup>5</sup> Ablation of host blood-derived components by the donor immune system is usually not appreciated because donor stem cells engraft and eventually support peripheral blood counts. In addition, high-dose chemotherapy ablates such a large percentage of host marrow and lymphoid elements that we often do not recognize the ablative power of the donor immune system. Such ablation is, however, a clinically prominent feature of transfusion-associated GVHD, where marrow aplasia is often a proximal cause of death.

The increased expression of homing receptors for the GI tract on T cells shortly after BMT emphasizes the importance of these receptors, and the fact that T cells lacking homing receptors did not induce GVHD *in vivo* even though they responded vigorously to alloantigens *in vitro*. This discrepancy may be a partial explanation as

to why the mixed leukocyte reaction does not predict the incidence or severity of GVHD; if T cells can't traffic to sites of stimulation, they won't be stimulated. It also suggests hope for novel GVHD therapeutic strategies that paralyze T cells rather than simply choking them to death. ■

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and  $\alpha\beta$  CD8<sup>+</sup> T cells that, when engaged by its ligands, can activate a killer program. In 2002, Groh and colleagues<sup>1</sup> showed that a variety of common human cancers express ligands for NKG2D collectively called MHC class I chain related (MIC), which are not expressed on most normal tissues. MIC<sup>+</sup> tumors shed soluble MIC into the bloodstream, resulting in NKG2D endocytosis and marked reduction of its surface expression on large numbers of tumor-infiltrating and blood T cells, which severely impairs their responsiveness to tumor antigens.<sup>1</sup> When a tumor sheds a ligand that naturally binds to and down-modulates a killer cell's triggering receptor, Mother Nature is speaking loud and clear: certain tumors are evading the immune system, not by avoiding immune recognition but rather by using it to their advantage.

In the current issue of *Blood*, Boles and colleagues discover a new receptor-ligand story that sheds light on how a common human tumor, this time lung cancer, has evolved yet another mechanism to evade the cytotoxic arm of the immune system. Loss of heterozygosity plus gene methylation silences tumor suppressor in lung cancer-1, or *TSLC1*, that encodes a cell-surface protein called Necl-2. Necl-2 belongs to a family of nectin-like molecules required for cell-to-cell adhesion in ordered epithelial-cell growth. The absence of Necl-2 likely contributes to loss of cell polarity and cell-to-cell adhesion during neoplastic transformation and metastasis. Boles and colleagues have uncovered yet another very important reason for Necl-2 to be silenced in cancer: it serves as the ligand for a receptor only expressed on activated NK cells and CD8<sup>+</sup> T cells. The receptor, known as class I-restricted T-cell-associated molecule, or CRTAM, is abundantly unregulated on NK cells following activation of specific receptors (eg, CD16 or NKp30) or following incubation with NK tumor-cell targets such as K562. Likewise, engagement of CD3, which might occur in T-cell recognition of a tumor antigen, up-regulates CRTAM in CD8<sup>+</sup> cytotoxic T lymphocytes (CTLs). In a series of elegant experiments, these researchers show that engagement of CRTAM by Necl-2 promotes cytotoxicity by NK cells and interferon-gamma (IFN- $\gamma$ ) secretion by CD8<sup>+</sup> CTLs. Importantly, they also demonstrate that when Necl-2 is expressed by

#### ● ● ● PLENARY PAPER

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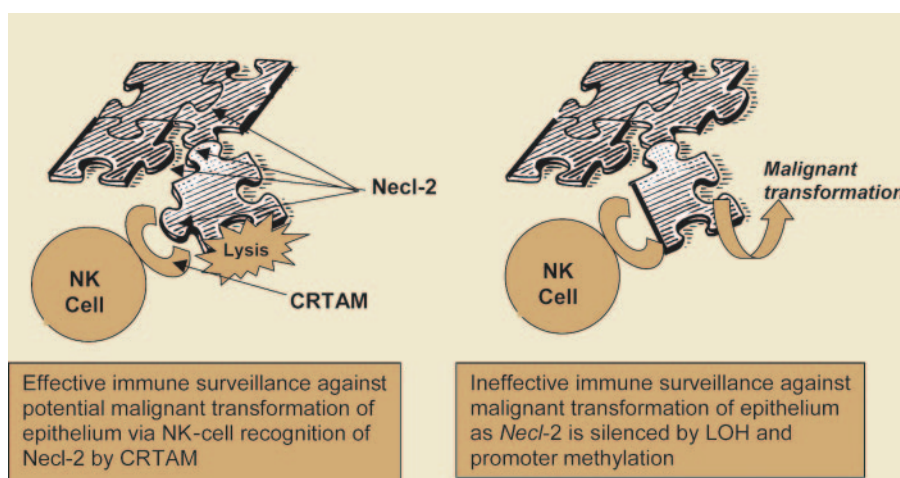
## Immune surveillance against common cancers: the great escape

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Cancer cells easily triumph over the body's defenses. Gene fusion, gene amplification, and gene silencing promote tumor survival, proliferation, and angiogenesis. Escape from immune surveillance adds another piece to the cancer puzzle.

**M**other Nature has revealed few clues that support the notion that common human cancers pay attention to the immune

system. Natural killer group 2D (NKG2D) is a c-lectin-like receptor on the surface of human natural killer (NK) cells,  $\gamma\delta$  T cells,



Silent escape.