Comment on Bagnara et al, page 5463

Two-faced T cells in CLL

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In this issue of Blood, Bagnara et al describe the development of a reliable and convincing xenograft model of CLL that recapitulates aspects of the leukemic microenvironment and gives intriguing insights into disease biology.1

Animal models of leukemia fall into 2 categories. There are those in which the disease is induced in cells of the host, usually a mouse, by genetic modification of the appropriate stem cell population. Xenograft models, on the other hand, involve the direct transfer of human leukemia cells into immunodeficient mice. They have the advantage of not requiring a detailed knowledge of pathogenesis but, because all nonmalignant cells are of mouse origin, often fail to provide the correct tumor microenvironment. After many years without a convincing animal model of either type, CLL researchers now seem to have both.

Transgenic mice that overexpress the human T cell leukemia-1 oncogene, or with deletions of miR15a/16.1 have both been used as models of CLL.2,3 In each case the resulting disorder closely resembles CLL, however only the latter involves a gene that is abnormal in the human disease. Now, Bagnara and colleagues appear to have solved the CLL xenograft problem. They reasoned that the explanation for the previous failure to engraft immunodeficient mice with human CLL was the lack of an appropriate microenvironment, rather than rejection by the host. In an elegant series of experiments they demonstrate that activated autologous CD4+ T cells are all that is required for the proliferation and engraftment of CLL in these animals. Intriguingly, after approximately 3 months the CLL disappeared, coincident with the onset of lethal graft-versus-host disease (GVHD), suggesting that the T-cell compartment in CLL contains subpopulations that are capable of promoting and eradicating the disease.

Previous attempts at developing a CLL xenograft model used nonobese diabetic severe combined immunodeficient mice (NS), which have profound defects of T- and B-cell function but intact Natural Killer (NK) immunity. In these animals, CLL cells rapidly disappear from the peripheral blood after intravenous injection, most likely because of NK effects, but persist without evidence of proliferation after administration into the peritoneal cavity.4 By combining both routes, Durig and colleagues showed that tumor cells from patients with more aggressive CLL can engraft and proliferate in the spleen whereas early-stage disease predominantly gives rise to T-cell engraftment.5 Similar results were observed in radiation chimeras,6 leading to speculation that CLL T cells may have suppressive or stimulatory effects depending on the disease stage.

In an effort to improve engraftment, Bagnara et al7 used NSG mice, which additionally lack the IL2Rγ subunit and have deficient NK function. They also preconditioned their recipients with various components of the human CLL microenvironment. Previous in vitro studies have identified a range of cell types of hematopoietic and mesenchymal origin as being of potential importance in this regard including vascular endothelium,7 T cells,8 myelomonocytic nurse-like cells,9 and bone marrow stroma.10 They therefore administered allogeneic umbilical cord CD34+ cells...
and mesenchymal stem cells (MSCs) to irradiated NSG recipients. Animals showing human hematopoiesis after 2 weeks were then given CLL peripheral blood mononuclear cells labeled with a cell division tracking dye.

The results were striking. In all cases in which significant proliferation of T cells was observed, there was robust proliferation and engraftment of the CLL. The presence of human MSCs had no impact, suggesting that murine mesenchymal cells can adequately support CLL growth. There was a direct correlation between the degree of proliferation of T cells and that of the CLL clone, which was prevented by the administration of antibodies to CD3 or CD4 but not CD8. Molecular analysis of the proliferated T cells demonstrated that they originated from the CLL patient and not the allogeneic cord blood CD34 cells. Similarly, immunoglobulin gene sequencing confirmed that the proliferating B cells were derived from the CLL clone. Epstein-Barr virus–driven proliferation, which has been an issue in other models, was not a significant factor.

Based on the observation that CD4 T–cell proliferation was required for engraftment and proliferation of the CLL, further studies were performed to refine the model. Allogeneic antigen presenting cells (APCs) including B cells and monocytes were substituted for the cord blood CD34 cells, and in both cases similar levels of T- and CLL-cell proliferation resulted. CLL cells were present in the bone marrow and spleen where structures resembling pseudofollicles containing blood vessels and activated CD4+ T cells were found. After approximately 12 weeks, the mice developed fatal GVHD, which was preceded by loss of both allogeneic APCs and autologous CLL cells (see figure).

So what is the significance of these findings? First, after almost 20 years of trying, we now have a convincing xenograft model of CLL that will be useful for both investigating disease biology and evaluating novel therapies. This is particularly timely given current difficulties in performing clinical trials and the multiplicity of targeted agents that are now becoming available.

Perhaps the main significance of this work, however, lies in the many questions it raises about the role of T cells in CLL. It is clear that in this model the presence of activated CD4+ T cells is required for tumor proliferation, but does this also apply to the human disease? Probably it does, because activated T cells are present in lymph node pseudofollicles from patients with CLL where they are in contact with proliferating tumor cells. How does this happen given the fact that CLL cells inhibit T-cell activation? In the present study a very strong and nonphysiologic activation signal, in the form of an in vivo allogeneic mixed lymphocyte reaction, was supplied; but what causes T-cell activation in the human disease? Is it an autoantigen, a tumor antigen, or even an infection as is the case in some marginal zone lymphomas? As much as T cells facilitate the development of CLL in the mouse, they also ultimately prove to be its undoing. By 12 weeks, mice engrafted with CLL and third-party APCs develop GVHD, and both the APCs and the CLL are eliminated. Is this a nonspecific phenomenon occurring in the face of the immunologic storm that is GVHD or have T cells with antileukemic activity been uncovered, as previously suggested by others? While clearly capable of further development, for example, by removing the requirement for allogeneic stimulation, this system should allow these questions and many more to be addressed.

**Conflict-of-interest disclosure:** The author declares no competing financial interests.

**REFERENCES**


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**CLINICAL TRIALS**

Comment on Straus et al, page 5314

**Hodgkin lymphoma: answers take time!**

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In this issue of *Blood*, Straus and colleagues on behalf of the Cancer and Leukemia Group B (CALGB) present the outcome of a phase 2 trial of doxorubicin, vinblastine, and dacarbazine for patients with early-stage, nonbulky, Hodgkin lymphoma. The complete response rate and progression-free survival were inferior to comparable series, emphasizing the challenges of improving outcome in this highly curable population.

The treatment of Hodgkin lymphoma, particularly in early stage, has been one of the true victories in the war on cancer. The current standard therapeutic approach of 2-4 cycles of doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) (depending on clinical risk factors) followed by 20-30 Gy of involved field radiation therapy definitively cures > 90% of patients. Even for patients with adverse clinical prognostic features, 5-year progression–free survival approaches 90% with this combined modality approach. Unfortunately, these cures have come with a cost. In an analysis of > 1000 patients under age 50 treated with radiation (with or without chemotherapy) between 1967 and 1997 for early-stage Hodgkin lymphoma, the relative risk of mortality from all causes,
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