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NEOPLASIA

Comment on Bassing et al, page 2163

RAGs found "not guilty": cleared by DNA evidence

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A recent paper from the Alt laboratory shows that recombination activating genes (RAGs) are not responsible for double-strand DNA breaks associated with some chromosomal translocations in pre–T-cell lymphomas.

B- and T-cell lymphomas and leukemias are frequent hosts to chromosomal translocations, duplications, and deletions, the best studied of which are known to result in the hyperactivation of oncogenes or the fusion of 2 genes into a chimeric oncogene. How do these DNA-rearranging events occur? For the most part, there are 2 basic requirements: 2 distant double-strand DNA breakages, and joining of the wrong ends together. A fair amount of circumstantial evidence implicates the recombination activating gene (RAG) proteins, RAG-1 and RAG-2, as key suspects in the DNA breakage step for some of these translocations. This is because at the pre-T and pre-B stages of development, RAGs naturally cut the DNA at the T-cell receptor (TCR) and B-cell receptor (BCR or immunoglobulin) loci as part of V(D)J recombination. In this physiologic process, RAGs cleave the DNA adjacent to segments encoding the various parts of the receptor, and the segments are stitched together into a functional receptor gene. The inherent imprecision in this process, in addition to the combinatorial possibilities of the segments that can be used, is the basis for much of the diversity seen in the adaptive immune system. However, pathologic consequences occur when RAGs cut other loci and join them aberrantly. Biochemical studies using purified recombinant RAG protein complexes have shown that their minimal target is the DNA sequence CACA, which occurs millions of times in the typical 6-billion base pair human nucleus. Thus, it has long been suspected that RAGs may be responsible for many of the chromosomal abnormalities in lymphocytes.

H2ax$^{-/-}$ p53$^{-/-}$ mice, which typically die from pre-T-cell lymphomas with clonal chromosomal defects, offer an opportunity to test that hypothesis. A significant proportion of the T-cells of H2ax$^{-/-}$ mice contain translocations, but for the most part they do not develop lymphomas. On the other hand, p53$^{-/-}$ and p53$^{-/-}$ RAG2$^{-/-}$ mice die from pre–T-cell lymphomas, but mostly without clonal translocations, deletions, or duplications. Could the chromosomal abnormalities seen in the lymphomas of H2ax$^{-/-}$ p53$^{-/-}$ mice be due to RAGs? In this issue of *Blood*, Bassing and colleagues find that H2ax$^{-/-}$ p53$^{-/-}$ RAG2$^{-/-}$ mice still die from pre-T lymphomas and that these lymphomas still have clonal chromosomal defects. Thus, the RAGs are probably not responsible for these translocations. It would appear that loss of p53 is responsible for the lymphoma and loss of H2ax is responsible for the translocations. So, going back to the translocation paradigm, what is causing the double-strand breaks if not RAGs? And how might H2ax prevent these translocations? These are questions for another study. It is worth noting, however, that in actual patients, a significant proportion of translocations may still be mediated by the RAGs, while in this system loss of H2ax might drastically increase the proportion of translocations by other mechanisms. Still, this study does open the door to the possibility that these other mechanisms may operate to the extent that H2ax—as well as the other components along the same or similar pathways—might fail.

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IMMUNOBIOLOGY

Comment on Crompton et al, page 2053

Attack of the CD4 clones

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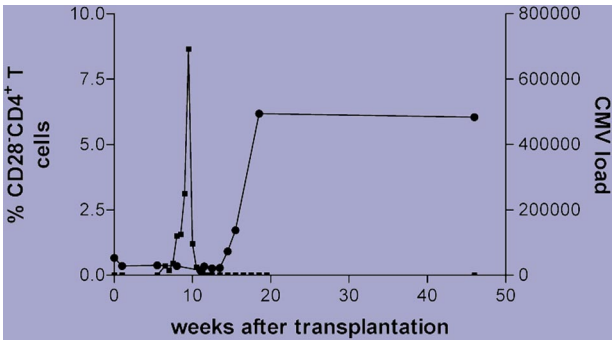
Latent human cytomegalovirus (CMV) infection is associated with vast clonal expansions of cytotoxic CD8$^+$ and CD4$^+$ T cells.

The human persistent herpes virus CMV has evolved in close relationship with its host, during which it has developed a wide variety of strategies to escape the immune system, which in its turn has responded with a refined series of defense lines. Latent CMV infection is accompanied by an increase in the number of circulating resting, effector-type CD4$^+$ and CD8$^+$ T cells with constitutive cytolytic activity. Although it is yet unsettled whether these cells are all CMV specific, it has become clear that T-cell responses to CMV are among the broadest and strongest analyzed so far, occupying a considerable fraction of the T-cell compartment.[1]

Cytotoxic CD28$^-$CD4$^+$ T cells emerge during primary CMV infection just following the decrease in viral load (see figure). Importantly, a considerable fraction of these cells can lyse CMV-antigen–expressing target cells, restricted by HLA class II. The CMV-specific cytotoxic CD28$^-$CD4$^+$ T cells present during latency were shown to have developed, through very strong selection, from the virus-specific cells early in primary infection.[2]



Longitudinal course of CD28⁻CD4⁺ T cells in relation to CMV viral load after transplantation of a kidney from a CMV-seropositive donor into a CMV-seronegative recipient. Squares indicate viral load; circles, percentage of CD28⁻CD4⁺ T cells in peripheral blood.

In this issue of *Blood*, Crompton and colleagues describe the cytotoxic CD4$^+$ T-cell response specific to the DYS peptide (DYSNTHSTRYV) from the CMV glycoprotein B (gB), restricted through HLA-DRB*0701. They show that this response constitutes a very large proportion of the total CMV-specific CD4$^+$ T-cell response in HLA-DRB*0701 individuals. Remarkably, their study reveals a clear immunodominance for this CD4$^+$ immune response, as reflected by a striking conservation of T-cell receptor (TCR) sequence between different individuals. In that respect, it resembles the CMV-specific CD8$^+$ immune response, which is characterized by a high degree of clonal selection in vivo.[3] Crompton et al also show that TCR usage by these CMV-gB–specific CD4$^+$ T cells is highly homologous to that in HLA-DRB*0701 patients with monoclonal TCR$\alpha\beta^+$CD4$^+$ T–large granular lymphocyte (T-LGL) lymphocytosis,[4] strongly suggesting that the CMV-gB peptide may be one of the driving forces behind this clonal expansion. Still, in light of the high prevalence of CMV infection in humans (about 75%) and the rather frequent prevalence of HLA-DRB1*0701 (about 30%) in the white population, the rare occurrence of these monoclonal expansions implies that additional factors are involved in the pathogenesis of CD4$^+$ T-LGL.

The immune response toward CMV is aimed at maintaining latency in infected individuals, but recent studies, like the present one, indicate that this response nevertheless occurs at the expense of the heterogeneity of the T-cell repertoire. Moreover, a huge amount of potential harmful effector cells is generated, which may produce proinflammatory cytokines and execute cytotoxicity that can cause substantial collateral damage in excess of what is needed to suppress the virus. Thus, design of a vaccine or immunotherapy against CMV should include the correlates of a protective immune response against the virus with avoidance of induction of the strong effector T-cell increase as seen in natural CMV infection.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● CLINICAL OBSERVATIONS

Comment on Abdelkefi et al, page 1805

Is tandem autotransplantation necessary in myeloma?

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In this issue of *Blood*, Abdelkefi and coworkers use a randomized trial to show that the immunomodulatory agent thalidomide, applied after a single transplantation for 6 months, results in improved response rates and better progression-free and overall survival (OS) compared with tandem transplantation.

Recent availability of novel agents for the treatment of myeloma has resulted in exploration of their use in several different clinical settings.[1-3] Abdelkefi and colleagues have attempted to replace high-dose melphalan with a modest course of thalidomide (100 mg daily for 6 months). The provocative results of their study raise the question of whether effective maintenance therapy after one transplant obviates the need for tandem transplantation.

However, the study does raise some questions. The 3-year survival of patients in the tandem transplantation arm is similar to that in the Intergroupe Francophone du Myélome (IFM)–96 trial, but significantly inferior to that in the Bologna-96 study.[4,5] However, it is remarkable that the 3-year 88% survival of patients in the single transplantation arm is remarkably better than in the single transplantation arms of the IFM and Bologna studies. Can such differences be attributable solely to thalidomide?

While thalidomide was available as effective salvage therapy for patients relapsing after tandem transplantation, other effective agents such as bortezomib or lenalidomide were not available. How many patients from each arm relapsed, and what their responses were to salvage therapy, is rather difficult to discern from this paper. The fact that 15 of the 18 patients who received thalidomide for relapse died of progressive disease in a relatively short period of time is unusual. In the long-term follow-up of their original observation, Barlogie et al[1] showed almost 50% of patients were alive at 2 years following thalidomide monotherapy. In the era of effective salvage therapy, it is rather unusual to see virtually identical event-free survival and OS in each arm of the study.

This study, although provocative, needs confirmation in a setting where other appropriate salvage therapy options are available to the patients. There are several ongoing clinical trials exploring the role of novel agents in the maintenance setting. Until more data are available, second transplantation should not be abandoned.

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

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