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Response:

Predicting response to ATG for patients with myelodysplastic syndrome

Madkaikar and Ghosh raise important points about the cost and toxicity of antithymocyte globulin (ATG) and thereby underscore one of the conclusions of our recently terminated clinical trial.[1] However, we feel that the use of ATG and similar therapies for some patients with myelodysplastic syndrome (MDS) should not be dismissed prematurely. While the series of ATG-treated MDS patients recently reported by Molldrem et al (the largest to date) may include individuals atypical of those commonly encountered in community clinical practice (eg, selected by their motivation to participate in a clinical trial and ability to travel to a referral center for therapy), the response rate observed (34% red cell transfusion independence, with improvement in approximately half of patients with severe thrombocytopenia and neutropenia) suggests that there is indeed a subset of MDS patients whose ineffective hematopoiesis is at least partially immune mediated.[2] Such patients may attain palliative benefit from ATG or other forms of immunomodulatory therapy. The challenge to clinicians is identifying these individuals prospectively, so that the expense and toxicity of the drug can be avoided in patients who cannot be expected to respond—especially in settings where health care resources are limited.

MDS is an extraordinarily heterogeneous disorder, and patients seen in the clinic with this catch-all diagnosis likely suffer from a very diverse set of maladies.[3] Recently, Sauntharajah et al devised a method based on a logistical regression model to predict which patients classified as having MDS are most likely to respond to ATG.[4] Important positive predictive factors in this analysis are younger age, shorter duration of red cell transfusion dependence, and HLA-DR15 (DR2) positivity. These surrogate markers for immune-mediated marrow failure will need to be validated in a prospectively treated cohort of MDS patients, and we can expect refinement of the model as experience with the drug accumulates.

Madkaikar and Ghosh also raise the issue that 6 of the 8 nonresponding patients in our series had refractory anemia with excess blasts (RAEB). Indeed, Molldrem et al demonstrated that patients with refractory anemia (RA) are much more likely to respond to ATG than those with RAEB.[2] This is not surprising; in the chronic myeloid disorders, it is suspected that by the time marrow myeloblasts begin to accumulate, multiple genetic injuries have occurred at the level of progenitor cells and the immune system is no longer likely to be a major player in sustaining ineffective hematopoiesis. Notably, however, all of the patients in our series had 10% blasts or less, and the International Workshop on Prognostic Factors in MDS identified 10% blasts as an important predictor of survival and rate of leukemic transformation.[5] The "10% cutoff" separating low-risk from high-risk disease has been confirmed by others and is reflected in the new World Health Organization (WHO) classification of myeloid neoplasms that subdivides RAEB into 2 subtypes, RAEB-1 and RAEB-2, with 10% blasts as the distinction between them.[6] The ATG trial of Molldrem et al preceded the WHO classification and the recently reported British antilymphocyte globulin (ALG) trial[7] also used the older French-American-British MDS classification, so it is not clear how refractory cytopenia with multilineage dysplasia patients (RCMD—another new WHO category[6]) will respond to ATG/ALG compared with RA patients, nor is it known whether RAEB-1 patients might fare better than RAEB-2.

Finally, we note that several different types of ATG and antilymphocyte globulin (including those derived from horses, rabbits, and goats) are currently available for use around the world and that the response patterns to one preparation may differ from those observed with another.

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To the editor:

Continuous flow leukapheresis induces expression of stress genes in lymphocytes: impact on microarray analyses

DNA microarray technology offers new means to investigate gene expression patterns in health and disease.[1-3] We recently used DNA microarray technology to investigate gene expression in lymphocytes of 3 groups of individuals: HIV-infected patients with detectable plasma viremia (viremic) and undetectable plasma viremia (aviremic), and HIV-negative donors (S.M., C.E.B., A.S.F., unpublished observations, 2003). The pattern and nature of genes found to be up-regulated in the latter group relative to the other 2

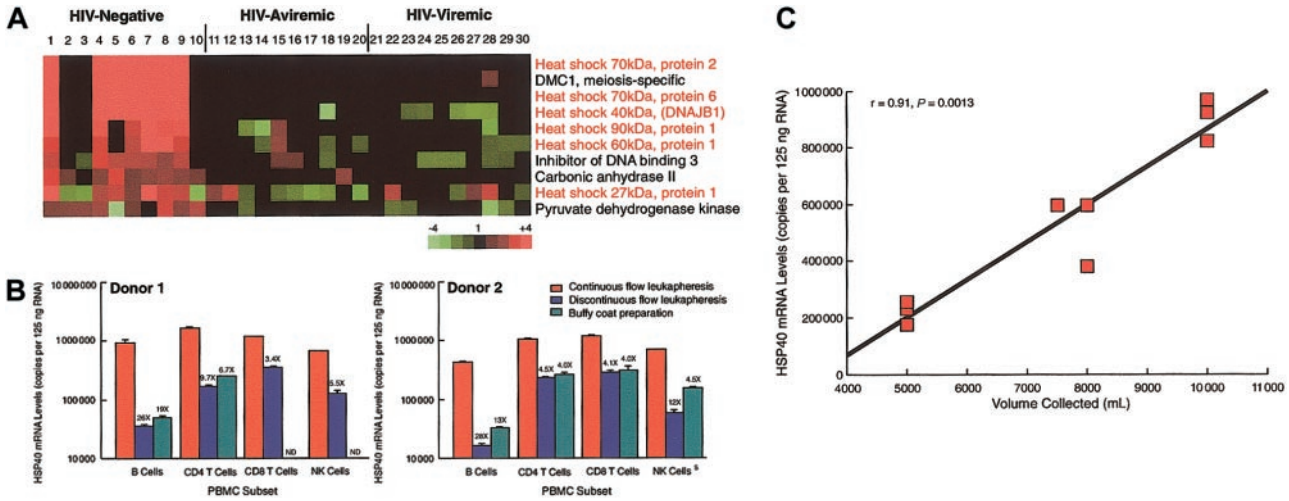

Figure 1. Gene expression profiles in lymphocytes. (A) cDNA microarray analyses[4] on B cells isolated from peripheral blood lymphocytes. (B) Real-time reverse transcription–polymerase chain reaction (RT-PCR) analysis[5] of HSP40 expression in lymphocytes obtained by CFL, DFL, and BC procedures. Data are mean ± SD of triplicates and numbers above the data bars represent fold differences relative to CFL values. ND indicates not done. (C) Correlation between copies of HSP40 mRNA measured in B cells of HIV-negative donors and volume of blood collected by CFL.

groups (Figure 1A) suggested that the method used to collect the samples might have been responsible for the differences observed. Nearly two thirds of the genes in this cluster were heat shock proteins (HSPs), recently shown to be up-regulated by mechanical stress,[6] and 3 of the HIV-negative donors (donors 2, 3, and 10) had profiles that appeared to be more closely related to the profiles of both groups of HIV-infected patients. A retrospective analysis revealed that the blood products collected from these 3 outliers were buffy coat (BC) preparations, whereas the blood products from the other 7 HIV-negative donors were obtained from continuous flow leukapheresis (CFL) using a Baxter Healthcare CS-3000 Plus device (Deerfield, IL). Considering that the cells obtained from HIV-infected patients were processed by a third procedure, namely discontinuous flow leukapheresis (DFL) using a Haemonetics MCS+ device (Braintree, MA), we evaluated the effect of blood collection procedures on HSP expression by subjecting 2 HIV-negative donors to all 3 procedures. HSP40 and HSP90, 2 representative genes from the microarray analyses, were chosen for validation by quantitative RT-PCR. Measurements were made on B cells, as well as in natural killer (NK) cells, and $CD4^+$ and $CD8^+$ T cells.

CFL induced a dramatic increase in mRNA levels of HSP40 in B cells compared with BC and DFL preparations (Figure 1B). While not as dramatic, similar trends were observed in all 3 other lymphocyte populations investigated. Levels of HSP90 mRNA were also found to be up-regulated in samples derived from CFL compared with BC and DFL procedures (data not shown). These data demonstrate that CFL is associated with sharp increases in HSP gene expression and that B cells are the most profoundly affected among the lymphocyte populations.

The main difference between the 3 methods of collecting blood products is centrifugation time; cells undergo 90 to 180 minutes of centrifugation by CFL compared with 10 minutes by DFL and 6 minutes by BC. A direct association between time of centrifugation and HSP gene induction was confirmed by showing a direct and highly significant correlation between volume of blood collected by CFL, processed at a rate of approximately 55 mL/min, and levels of HSP40 mRNA measured in B cells (Figure 1C).

Continuous flow devices are routinely used, especially when resources are a limiting factor, to provide adequate quantities of mononuclear cells for research or therapeutic use. However, these devices may lead to confounding results in microarray and real-time RT-PCR analyses, especially when CFL-derived samples are compared with samples derived from HIV-infected and cancer patient populations, where DFL and BC procedures are favored. In this setting, the optimal device for collecting larger numbers of cells may be a continuous flow cell separator in which the collected component does not remain within the centrifugal field for the entire duration of the procedure.

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