To the Editor:

Two studies published recently in Blood and Transfusion describe application of an inbred mouse model of allogeneic transfusion to investigate the role of donor leukocytes in inducing immunosuppressive consequences of transfusion. Blajchman et al showed a median fivefold increase in the frequency of pulmonary metastases at 21 days in C57/Bl/J6 (H-2b) mice that were transfused with allogeneic (Balb/c [H-2d]) whole blood 10 days before injection of a fibrosarcoma cell line, relative to the rate of metastases in mice pretransfused with either syngeneic or leukodepleted allogeneic blood. Moreover, spleen cells harvested from C57/Bl/J6 recipient mice 10 days after allogeneic transfusion and infused into other C57/Bl/J6 mice could passively transfer the tumor growth-promoting effect. Gianotti et al, in studies of the effect of allogeneic transfusions on sepsis-related mortality, infused either leukocytes, red blood cells, or plasma from C3H/HeJ (H-2k) mice into Balb/c (H-2d) mice. Five days later the mice were subjected to a 20% burn and gavage with $1 \times 10^{10}$ Escherichia coli. The percentage of viable bacteria and the mortality rate were significantly higher in the group receiving allogeneic leukocytes relative to other allogeneic blood constituents or control (buffer or syngeneic blood) infusions.

These data further implicate donor leukocytes as the primary culprit in transfusion-related complications, thereby lending additional support to the movement toward routine leukodepletion of cellular blood components. Although our own research also implicates allogeneic leukocytes in transfusion-induced immunosuppression, recent studies in our laboratory characterizing survival kinetics of donor leukocytes in transfused humans, dogs, and mice indicate that allogeneic transfusions between strains of inbred mice may not be a good model system for such investigations.

To facilitate survival studies of transfused leukocytes, we developed quantitative, allele-specific polymerase chain reaction (PCR) assays directed at Y chromosome-specific sequences of humans, dogs, and mice. Based on in vitro mixing studies of male into female blood for each species, the assays detect as few as 1 to 5 male donor leukocytes per 50 µL of recipient blood containing $10^3$ to $10^6$ female leukocytes. Quantity is achieved by parallel amplification and autoradiographic image analysis of dilutions of male into female blood. Samples with $Y$-specific signal exceeding the dynamic range of the assay are serially diluted and reevaluated.

In preliminary studies involving four transfused orthopedic surgery patients and four outbred dogs, we observed greater than 99.9% clearance of infused allogeneic leukocytes within 48 hours of transfusion, and male donor cells were undetectable by 7 days posttransfusion (Fig 1C). In contrast, we were surprised to find that in inbred mice, allogeneic donor leukocytes are not cleared from the recipient’s circulation for weeks to months posttransfusion. Specifically, 150 µL (1/10 blood volume) of male C57B (H-2b) mouse blood was transfused into each of three female Balb/c (H-2d) recipients, and three female C57B recipients were given male Balb/c blood. Recipient mice were sampled daily for 1 week, and then at 3 weeks, 5 weeks, and 3 months. In all cases, male donor cells persisted at concentrations in the 1 to 10 cells/µL range (0.1% to 1% of total leukocytes) in the circulation of recipient mice for over 1 month posttransfusion, and donor cells were not cleared to undetectable levels (<1 cell/50 µL) until 3 months (Fig 1, A and B). Similar results were obtained after transfusion of male C3H (H-2k) blood into female Balb/c recipients. Despite this persistence of allogeneic donor cells, there was no evidence of graft-versus-host disease in recipient mice.

The failure of rapid clearance of donor cells after allogeneic transfusions of immunocompetent inbred mice was unexpected. Although low-level chimeras have been reported after bone marrow and solid organ transplantation of inbred mice and humans subjected to immunosuppressive regimens, inherent responsiveness to "non-self" HLA antigens of immunocompetent subjects should,
theoretically, lead to rapid donor cell clearance. We hypothesize that nonclearance may be due to the fact that the mice are genetically inbred and have been reared exclusively in haplotype identical colonies, and consequently that they have not had immunologic experience with nonself antigens. We are now performing transfusion studies with outbred mice, and with inbred mice that have been primed to the allogeneic strain by prior transfusions. Alternatively, the major histocompatibility complex (MHC) antigens expressed at high levels on murine (but poorly on human) red blood cells could adsorb anti-MHC antibodies, thereby precluding complete donor leukocyte clearance, and possibly facilitating induction of tolerance. This hypothesis can be investigated by comparing survival of transfused leukocytes with and without accompanying red blood cells.

In any event, our results raise concerns about extrapolation of results from transfusion studies involving MHC-mismatched inbred mice, to the situation in outbred humans. The “passive transfer” experiments of Blajchman et al require particular reevaluation: because the transfused inbred mice still contain large numbers of circulating allogeneic cells at 10 days posttransfusion, the transfusion of splenocytes from these mice into syngeneic mice may represent secondary transfusions of allogeneic leukocytes, rather than transfusions of “educated” syngeneic cells.

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REFERENCES


RESPONSE

Busch et al have devised an interesting technique for the detection of male leukocytes in the circulation of transfused females. This use of the allele-specific polymerase chain reaction using Y chromosome-specific sequences should prove to be a useful technique for detecting allogeneic leukocytes in some patients suspected of having graft-versus-host disease. We agree that their preliminary studies with allogeneically transfused inbred mice are rather surprising and need confirmation.

However, we do not agree that these observations cast doubt on the interpretation of the data contained in our recent article in which we reported, in both inbred (mice) and outbred (rabbits) animals, that the infusion of allogeneic blood was associated with an increase in the number of pulmonary metastases observed. Our experimental animal studies were performed in both inbred and outbred species precisely because of concern about interpretation of data from only inbred animals. The results from both species were identical! Moreover, because the tumors used were syngeneic to the recipient animals, the persistence of allogeneic blood donor leukocytes would be expected to inhibit rather than stimulate tumor growth.

The letter by Busch et al also raised concern about our passive transfer experiments. In the latter, we provided evidence that the intravenous infusion of spleen cells from allogeneically transfused animals transferred the tumor growth-promoting effect to untransfused syngeneic animals. The spleen cell transfer experiments were also performed in both the inbred (mice) and the outbred (rabbits) animals and again, the presence of allogeneic cells of blood donor origin in those experiments, as predicted by Busch et al, would be expected to inhibit tumor growth rather than stimulate such growth. Furthermore, we have obtained data recently which show that the spleen cell transfer effect is mediated by soluble products produced by the syngeneically transferred spleen cells. Specifically, we have shown that spleen cells from allogeneically transfused animals, when placed inside sterile diffusion chambers and inserted...
intraperitoneally into syngeneic mice, promote tumor growth in a manner similar to that seen when such spleen cells are injected intraperitoneally or intravenously (Blajchman MA, Singal DP, unpublished observations, May 1993). The sterile diffusion chambers used in these experiments allow only the soluble factors produced by the cells contained therein to be released. These latter data thus indicate that the tumor growth-promoting effect of allogeneic blood transfusion does not require the presence of donor leukocytes to mediate this effect.

Thus, while the approach of Busch et al may prove useful for tracking allogeneic donor leukocytes, their data do not negate the observations from our two experimental animal models (inbred and outbred) that allogeneic blood transfusions promote tumor growth and that this effect can be transferred to naive animals by spleen cells.

REFERENCES


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Use of an inbred mouse model system for studies of allogeneic transfusion-induced immunosuppression [letter; comment]

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