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Disruption of Iron Regulation after Radiation and Donor Cell Infusion

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Iron overload is common in patients undergoing hematopoietic cell transplantation (HCT). Peritransplant events, such as total body irradiation (TBI), and the effects of donor cell infusion may contribute to iron overload, in addition to disease-associated anemia and RBC transfusions. Using murine models we show complex time- and dose-dependent interactions of TBI and transplanted donor cells with expression patterns of iron regulatory genes in the liver. Infusion of allogeneic or syngeneic donor T lymphocytes increased serum iron, transiently up-regulated interleukin-6 (*IL-6*) and hepcidin (*Hamp*), and down-regulated ferroportin1 (*Fpn1*). After 7 to 14 days, however, changes were significant only with allogeneic cells. TBI (200 to 400 Gy) also induced *IL-6* and *Hamp* expression but had little effect on *Fpn1*. TBI combined with allogeneic donor cell infusion resulted in modest early up-regulation of *IL-6*, followed by a decline in *IL-6* levels and *Hamp* as well as *Fpn1*, and was accompanied by increased liver iron content. Injection of Fas ligand–deficient T lymphocytes from *gld* mice resulted in substantially lower alterations of gene expression than infusion of wild-type T cells. The agonistic anti-Fas antibody, JO2, triggered early up-regulation of *Stat3* and *IL-6*, followed by an increase in *Hamp* and decreased expression of *Fpn1* by 7 to 14 days, implicating Fas as a key modulator of gene expression in HCT. Minimal histologic changes were observed in mouse liver and duodenum. These data show profound and interacting effects of TBI and cell transplantation on the expression of iron regulatory genes in murine recipients. Alterations are largely related to induction of cytokines and Fas-dependent signals.

INTRODUCTION

Patients undergoing hematopoietic cell transplantation frequently show iron overload, primarily related to anemia due to the underlying disease and therapeutic RBC transfusions [1,2]. Little is known regarding the impact of the transplantation process itself or inflammatory changes associated with graft-versus-host disease (GVHD) on iron homeostasis. To investigate the impact of components of the transplant procedure on iron homeostasis, we previously conducted experiments in nonobese diabetic–severe combined immunodeficient NOD.CB17-*Prkdc*^{scid}/NcrCrl (NOD/SCID) mice and showed that allogeneic T cell infusion resulted in fluctuations in serum iron levels and increased liver iron content after transplantation. These results supported the notion that alloreactive T cells and T cell–dependent signals contribute to iron dysregulation [3]. Furthermore, cytotoxic transplant conditioning, such as total body irradiation (TBI), results in arrest of erythropoiesis, which is associated with a period of hyperferremia, increased transferrin-iron saturation, and appearance of toxic non–transferrin-iron-bound iron in the circulation [4-7]. Concurrently, tissue injury inflicted by cytotoxic transplant conditioning results in the release of cytokines, including IL-6, which would be expected to *up-regulate* hepcidin and oppose iron accumulation [8,9].

We showed in wild-type mice *not* subjected to transplant conditioning that signals initiated via the death receptor Fas (CD95, highly expressed on hepatocytes) by the agonistic anti-Fas antibody JO2 resulted in *down-regulation of hepcidin*,

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which involved *IL-6* and *Stat 3*, albeit in an unexpected direction [10]. Because allo-activated T cells up-regulate expression of the cognate Fas ligand, CD178, these observations were consistent with a T cell–mediated effect on iron homeostasis via Fas/Fas-ligand interactions. In further support of this hypothesis, overexpression of the Fas adaptor molecule FLIP-long, which *interferes* with Fas signaling, allowed for *maintenance of hepcidin levels* [11]. Based on that model, in the present study we characterized interactions between the effects of TBI conditioning and donor cell–dependent signals on iron homeostasis in wild-type mice. Because radiation potently modifies gene expression [12], we hypothesized that responses to transplanted donor cells would thereby be modified, resulting in altered effects on iron homeostasis.

METHODS

Reagents

T cell isolation kits for the positive selection or depletion of $CD8^+$ T cells (Ly-2, 130-049-401; 130-095-236) were obtained from Miltenyi Biotec (Auburn, CA). Cells were sorted by autoMACS Pro Separator (Miltenyi Biotec) according to the manufacturer's protocol. JO2, an agonistic hamster anti-mouse Fas monoclonal antibody, was purchased from BD Biosciences (San Diego, CA). Serum iron levels were measured using the Quanti-Chrom Iron Assay Kit (BioAssay Systems, Hayward, CA).

Mice

C57Bl/6 [H-2^b], Balb/c [H-2^d], C3H/He [H-2^k], A/J [H-2^a], and C3H/He-Fasl^{gld}[H-2^b] mice were purchased from Jackson Laboratories (Bar Harbor, ME). C57BL/6 × C3H [H-$2^{b/k}$] and Balb/c × A/J [H-$2^{d/a}$] F1 hybrid mice were bred at the animal facility of the Fred Hutchinson Cancer Research Center. Female mice were 6 to 8 weeks old at the time of experiments. All experiments were approved by the Fred Hutchinson Cancer Research Center Institutional Animal Care and Use Committee.

Transplantation Models

Transplants without recipient conditioning

Transplantation of T cells. Balb/c[H-2^d], C57 BL/6[H-2^b], or C3H/He-Fasl^{gld} [H-2^k] mice served as donors, and Balb/c × A/J and C57BL/6 × C3H mice served as recipients (P to F1 model). $CD8^+$ T lymphocytes were prepared from donor spleens by magnetic bead sorting (Miltenyi Biotec), and 1, 5, or 10×10^6 $CD8^+$ donor T lymphocytes were injected i.v. Syngeneic F1 into F1 transplants were carried out in parallel.

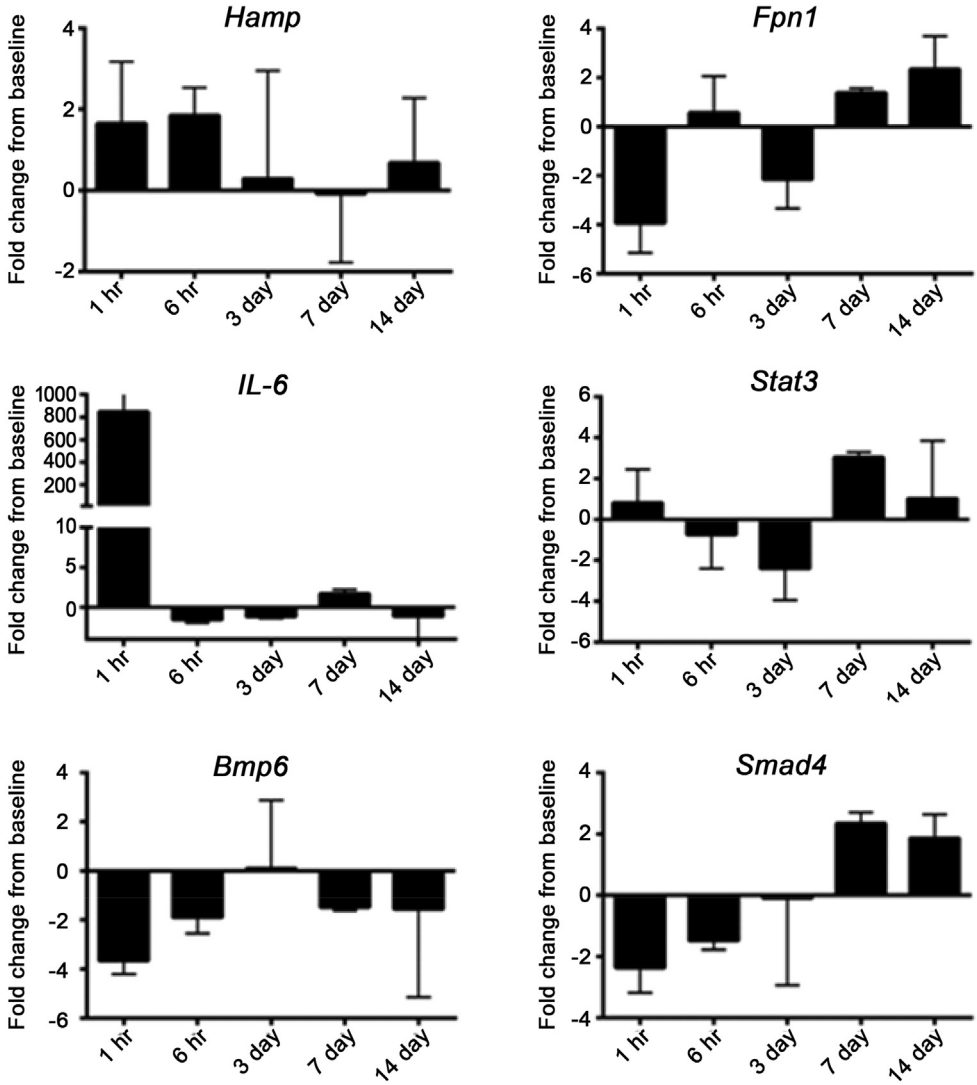

Figure 1. Expression of iron regulatory genes in mouse liver after allogeneic T cell infusion. Early up-regulation of *IL-6* was accompanied by increased expression of *Hamp* and down-regulation of *Fpn1* with little change in *Stat3* expression, suggesting the possibility of a direct effect of T cells on *Hamp*. *Bmp6* and *Smad4* declined early (as *Hamp* expression rose) before returning toward or above baseline. Gene expression in hepatocytes, as assessed by mRNA levels and determined by RT-PCR. Shown is the fold change compared with expression in unmodified controls determined at 1 hour to 14 days after i.v. injection of 1×10^6 allogeneic T cells (P→F1) (mean ± SEM of 3 experiments).