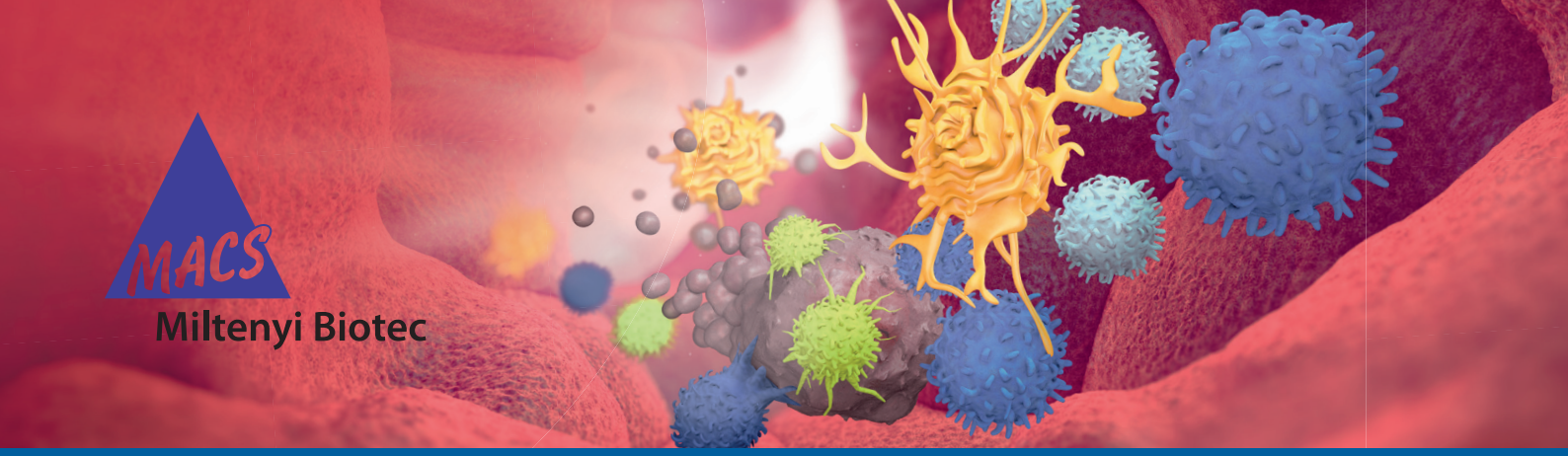


Allogenic T cell infusion alters iron regulatory gene expression

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Background

Iron overload is a common side effect in patients undergoing hematopoietic cell transplantation (HCT) and can be caused by total body irradiation (TBI) or by the effects of donor leukocyte infusion. Using murine models, Karoopongse and colleagues showed that TBI and cell transplantation change the expression patterns of iron regulatory genes in the liver, resulting in increased serum and liver iron content as well as in altered gene expression patterns of interleukin-6 (*IL-6*), hepcidin (*Hamp*), and ferroportin1 (*Fpn1*). Importantly, injection of Fas ligand–deficient T lymphocytes from *gld* mice does not lead to these gene expression alterations, implicating a key regulation role for Fas in transcription response in HCT.
To asses changes in the expression of iron regulatory genes, mouse CD8$^+$ T cells were isolated using the autoMACS® Pro Separator and subsequently injected into recipient mice. The effect of HCT on transcription in recipient hepatocytes was measured up to 14 days post injection using a real-time PCR (RT-PCR) based approach.

Materials and methods

Balb/c[H-2d] mice served as T cell donors. CD8$^+$ T lymphocytes were isolated from donor spleens by magnetic bead sorting using the autoMACS Pro Separator and CD8a (Ly-2) MicroBeads, mouse. 1×10^6 CD8$^+$ T cells were injected intravenously into C57BL/6 × C3H. One to 14 days post injection, hepatocytes were isolated. To assess the effects of T cell transfer on liver iron gene expression, total RNA was isolated from recipient hepatocytes and cDNA was synthesized using the µMACS One-Step cDNA Synthesis kit. *Hamp, Fpn1, Bmp6, Smad4, IL-6*, and *Stat3* expressions were determined by RT-PCR.

Results

Only one hour after injection, *IL-6* expression increased and *Hamp* expression followed (fig. 1). Few expression alterations were observed for *Stat3*. In contrast, *Fpn1* expression decreased directly after injection but rose again whereas *Hamp* expression dropped again to baseline level. *Bmp6* and *Smad4* expressions were reduced short after injection and increased in the tested timeframe to baseline or even higher levels.

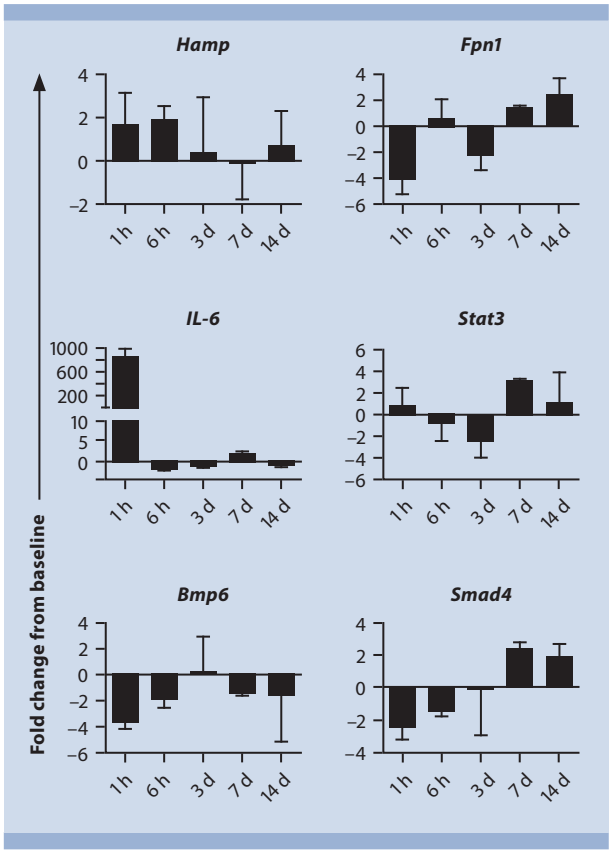


Figure 1: Expression changes of iron regulatory genes in mouse hepatocytes after allogeneic CD8$^+$ T cell infusion. Gene expression levels were normalized to expression levels of untreated mice (n=3, mean±SEM). Adapted from reference 1 with permission from Elsevier.

Conclusion

- The autoMACS® Pro Separator together with the CD8a (Ly-2) MicroBeads, mouse enabled efficient CD8$^+$ T cell isolation, suitable for allogenic infusion.
- CD8$^+$ T cell infusion alters iron regulatory gene expression in the recipient mouse in a Fas-dependent manner.

Product	Order no.
autoMACS® Pro Separator	130-092-545
CD8a (Ly-2) MicroBeads, mouse	130-117-044
µMACS One-Step cDNA Synthesis kit	130-091-902

Reference

[1] Karoopongse, E. *et al.* (2016) Disruption of iron regulation after radiation and donor cell Infusion. Biol Blood Marrow Transplant 22: 1173–1181. https://www.sciencedirect.com/science/article/pii/S1083879116300167

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