Fcγ receptor-positive cells play a critical role in rejection of allogeneic bone marrow cells mediated by anti-donor antibodies

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BACKGROUND

- Sensitization to major histocompatibility complex (MHC) antigens because of transfusion, pregnancy, and previous failed grafts is among the most critical challenges to clinical transplantation
- Sensitization increases the risk for bone marrow and solid organ graft rejection and sometimes causes patients to be excluded as candidates for transplantation
- A better understanding of the role that innate and adaptive immune responses play in allosensitization will allow a mechanistically driven approach to overcome sensitization in recipients

OBJECTIVES

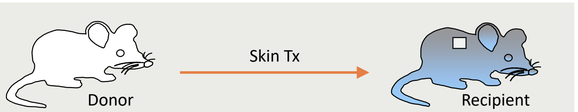
- To study the barrier of humoral immunity for allogeneic bone marrow engraftment in sensitized recipients
- To explore the role of T cells and Fcγ receptor-expressing innate immune cells in allogeneic bone marrow cell (BMC) rejection in sensitized recipients using TCRβ/δ double-knockout (TCRβ/δ-/-) and Fcγ receptor-deficient (FcγR-/-) mice

METHODS

Mouse strains: Male B6 (H-2^b), BALB/c (H-2^d), B6 background TCRβ/δ$^{-/-}$, and B6 background FcγR$^{-/-}$ mice were obtained from The Jackson Laboratory

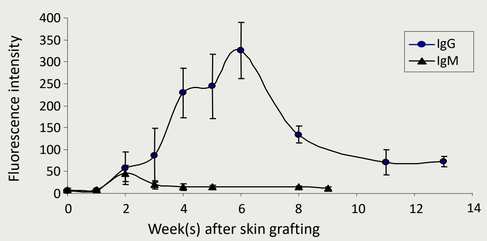
Flow cytometric cross-match (FCXM) assay: Donor-specific antibodies (DSA) were measured by FCXM. Sera were collected from sensitized mice weekly out to 12 weeks following sensitization. Splenocytes from naive donor mice were incubated with 5 µl of serum followed by FITC-conjugated polyclonal goat anti-mouse Ig, anti-mouse IgG, and anti-mouse IgM. Levels of circulating alloantibodies were assessed on a FACSCalibur by gating on the CD4$^+$ and CD8$^+$ T cell fraction. Presence of DSA was reported as mean fluorescence intensity (MFI)

Animal model



Sensitization of recipient animals was induced by transplanting skin grafts from MHC fully mismatched donors. Allogeneic skin grafts were rejected within 15 days for all animals. Tx, transplant.

Kinetics of IgM and IgG



After skin grafting, anti-donor IgM appeared early and peaked at week 2. IgM was nearly undetectable by week 4. Donor-specific IgG was detected starting at week 2 and peaked at week 6. IgG levels declined over time from peak levels but were maintained at a relatively high level beyond week 11. IgG, immunoglobulin G; IgM immunoglobulin M.

RESULTS

Alloengraftment in sensitized mice

Bone marrow transplant (BMT):
5–7 weeks after skin grafting sensitized mice were conditioned with 950 cGy TBI + different agents and transplanted with 80x10^6 BMC

Treatment*	Engrafted	Treatment*	Engrafted
-	0/6	Anti-αβ-TCR / CD8 + CyP	0/8
CyP	1/7	Anti-αβ-TCR / class I + CyP	0/4
Anti-CD8+CyP	2/6	Anti-CD8 / CD154	1/12
Anti-αβ-TCR	0/7	Anti-CD8 / CD154 / NK1.1	0/7
Anti-αβ-TCR + CyP	1/9	Anti-CD8 / CD154 + Fludara	0/4
Anti-αβ-TCR / CD8	0/3	Anti-CD8 / CD154 + Rapa	0/4
Anti-class II	0/3	Anti-CD8 / CD154 + Rapa + CyA	0/4
Anti-NK1.1	0/7	Rapa + CyA + Splenectomy	0/4
Anti-CD45RB	0/6	Rapa + CyA +anti-CD154	0/4
Anti-CD8 / CD45RB	0/7	Splenectomy+CyP+anti-CD8 / anti-CD154 +CyP	1/8
CVF	0/4	Anti-CD8 / CD154 + MMF	1/8
CVF+CyA	0/4	CVF+CyA+anti-CD154	0/6
MMF	0/4	Anti-B220	0/6
Rapa + CyA	0/6	3 x 80x10^6 BMCs	0/10

*No engraftment was achieved with 950 cGy TBI alone. Little improvement in alloengraftment was achieved in sensitized mice even with intensive lethal TBI plus other immune-based conditioning strategies. These results suggest that humoral immunity plays a critical role in graft rejection in sensitized mice.
BMCs, bone marrow cells; CVF, cobra venom factor; CyP, cyclophosphamide; CyA, cyclosporine A; MMF, mycophenolate mofetil; Rapa, rapamycin; Fludara, fludarabine.

Engraftment after hyperimmune serum transfer

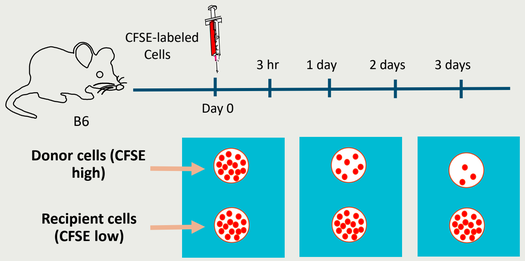
BMT: Naïve recipients + adoptively transferred sera
700 cGy total body irradiation (TBI) + BMC

Group	Serum Day -1	BMT Day 0	n	% Engrafted (28 days)
A	-	15×10^6	12	10/12
B	Naïve – 500 µl	15×10^6	8	7/8
C	Sen – 500 µl	15×10^6	7	0/7
D	Sen – 500 µl	40×10^6	8	0/8
E	Sen – 200 µl	15×10^6	4	0/4
F	Sen – 50 µl	15×10^6	7	0/7
G	Sen – 25 µl	15×10^6	5	1/5
H	Sen – 10 µl	15×10^6	6	5/6

Donor-specific antibodies led to donor BMC rejection. Hyperimmune serum was collected from animals with DSA Ig levels ≥ 276 MFI at 4-6 weeks after rejection of donor skin grafts. 10 – 500 µl of serum was intravenously injected into naive mice one day before TBI and allo-BMT from BALB/c donors. Passive sensitized serum transfer resulted in engraftment failure in all the animals with 500 to 50 µl serum (Group C – F). Only one out of 5 engrafted with 25 µl of serum infusion (Group G). Engraftment occurred in 5 out of 6 mice when 10 µl serum was injected to each mouse (Group H). In contrast, normal serum (500 µl) from naïve recipients did not prevent alloengraftment (Group B) compared with untreated group (Group A). BMT, bone marrow transplant.

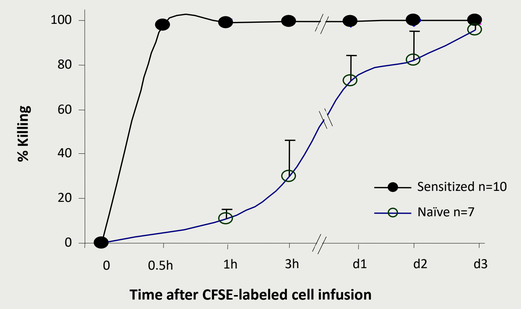
In vivo cytotoxicity assays

- 20x10^6 splenocytes from either donor target or recipient controls were labeled with different intensities of carboxyfluorescein succinimidyl ester (CFSE) and infused into experimental mice
- Peripheral blood was collected at selected time point to determine the rate of killing of donor cells by the ratio between donor and recipient cells



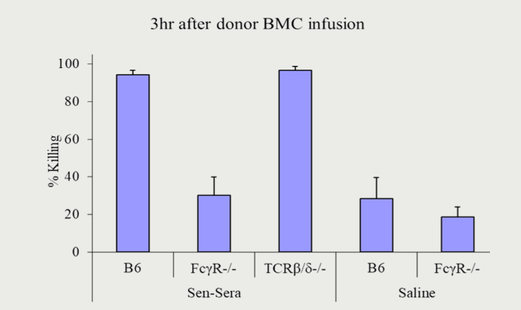
CFSE, carboxyfluorescein succinimidyl ester.

In vivo cytotoxicity assay in sensitized recipients



In vivo cytotoxicity assays were performed 5–7 weeks after sensitization. Sensitized recipients eliminated almost all donor cells within 0.5 hour, suggesting that sensitization plays a critical role in donor cell rejection in sensitized recipients.

Cytotoxicity assay in TCRβ/δ$^{-/-}$ and FcγR$^{-/-}$ mice



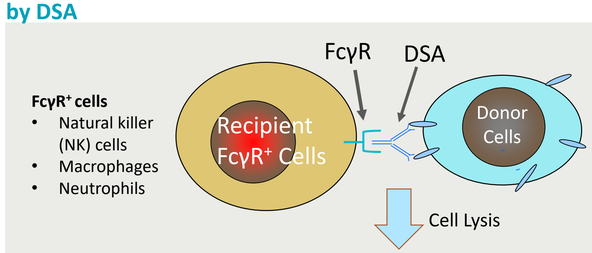
Methods

- The role of T cells and Fcγ receptor-expressing innate immune cells in allogeneic BMC rejection in recipients with DSA was explored using TCRβ/δ$^{-/-}$ and FcγR$^{-/-}$ mice
- Sera collected between 4-6 weeks after skin grafting were pooled from B6 mice sensitized by BALB/c skin grafts, and 300 µL was injected intravenously into naive TCRβ/δ$^{-/-}$, FcγR$^{-/-}$, and B6 mice
- In vivo cytotoxicity assays were performed 1 day after adoptive transfer of sensitized sera (sen-sera)
- Peripheral blood was collected at 3 hrs after CFSE labeled cell infusion to determine the rate of killing of donor cells

Results

- B6 control mice eliminated 28.5±11.0% of donor cells and B6 mice, with sen-sera rapidly killed 94.2±2.5% donor cells
- The percentage of cytotoxicity for TCRβ/δ$^{-/-}$ with sen-sera was 96.6±2.0%, which was similar to the percentage in B6 mice with sen-sera (P = 0.14)
- However, FcγR$^{-/-}$ with sen-sera only eliminated 30.2±9.7% of donor cells, which was similar to B6 controls (P = 0.82). The difference in cytotoxicity for FcγR$^{-/-}$ with sen-sera and TCRβ/δ$^{-/-}$ with sen-sera was highly significant (P = 0.00006)

The role of FcγR$^+$ cells in rejection of donor cells mediated by DSA



- Antibody-dependent cell-mediated cytotoxicity (ADCC): mostly by NK cells, which release perforin and granzyme
- Antibody-mediated phagocytosis: mostly by macrophages

CONCLUSIONS

- Humoral immunity is a primary barrier for allogeneic bone marrow engraftment in sensitized mice
- FcγR$^+$ cells, but not T cells, play a critical role in rejection of allogeneic BMC mediated by anti-donor antibodies
- Fc receptors are found on innate immune cells such as macrophages, neutrophils, and NK cells. Our data suggest that the immediate rejection of BMC in allosensitized recipients is driven by DSA-mediated phagocytosis by phagocytes and ADCC by NK cells
- Our study could have clinical significance for the management of BMC transplant patients to overcome the sensitization barrier

Acknowledgments

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Disclosures

HX and **YH** are employees of Talaris Therapeutics, Inc. **SI** has ownership interest in Talaris Therapeutics, of which she is Chief Scientific Officer and a Board Member.

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