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POSTER ABSTRACTS

701.EXPERIMENTAL TRANSPLANTATION: BASIC AND TRANSLATIONAL

Recipient Resident Macrophage Plays a Crucial Role in Acute Graft-Versus-Host-Disease after Allogeneic Stem Cell Transplantation By Regulation of Damage-Associated Molecular Pattern Phagocytosis

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Introduction
Evidence shows allogeneic hematopoietic stem cell transplantation (alloHSCT) provides life-sustaining therapy for many hematologic malignancies. Unfortunately, in many cases it is complicated by acute-graft-versus-host disease (aGVHD), a life-threatening sequela. Most interstitial tissues contain populations of resident macrophages. Multiple studies have investigated the role of infiltrating macrophages in aGVHD, but little is known about the role of resident macrophage in aGVHD. To address this issue, we adopt a LysM $^{cre/cre}$-Hif1a $^{flox/flox}$ mice (KO)as host mice with abnormal macrophage whose host resident macrophage remains unchanged early after allo-HSCT. KO mice received alloHSCT after Total body irradiation (TBI) from wild-type(WT) mice of BALB/c background. The neutrophil and circulating monocyte/macrophage were replaced by WT cells and the resident macrophage remains unchanged after this procedure. The grade of aGVHD and survival was observed.
Methods
Recipient mice are on the background of C57BL/6(H-2 ^{b}), and donor mice are wild-type BALB/c (H-2 ^{d}) mice. All mice used were 8-12 week old. All animal experiment protocols were approved. LysM $^{cre/cre}$ (CRE) mice were taken as control recipients. Mice were exposed to TBI split into two doses separated by 4h. KO and CRE C57/BL mice received 9 Gy TBI. 18h after irradiation, mice of two groups were injected i.v with 1×10^{7} BM cells and 1.5×10^{7} splenocytes isolated from WT BALB/c mice. aGVHD score and survival are monitored daily. Isolation of Kupffer subsets was performed as CD45 $^{+}$CD11b $^{+}$ Ly6G $^{-}$F4/80 $^{+}$, which from KO and CRE mice of GVHD model were taken to do single-cell sequencing assay. Survival curves were plotted by the Kaplan-Meier method. The statistical significance between group means was determined using t-test by Graphpad prism9.5. The level of significance was set at $P<0.05$.
Results
The mean survival of KO mice is 18.6 days (95%CI 14.06-22.6) post alloHSCT, while the mean survival of control mice is 37.6 days (95%CI 29.7-45.6)(n=8)(P <0.001). Weight loss (% of baseline weight on the day of TBI) starts to differ on day 14 post-HSCT, with the KO group of 101.7 and Control group of 84.9 (P <0.05). GVHD score starts to differ on day 10 post-HSCT, with the KO group of mean score 5.1 and control group 3.9 (P <0.05)(n=8) (Figure1). The GVHD score remains statistically different till day 24 post-HSCT. On day 24, all KO mice die while one control mouse live up to 58 days. The liver function presented by ALT is different on day 14 post-HSCT, KO group with mean ALT 209.0 U/L is higher than Control group whose mean ALT is 64.9U/L(n=4). On day 21, Liver GVHD pathology score of the KO group is 7, compared to 5.2 of the control group(n=6)(P<0.05). Thus, KO mice present with a more severe aGVHD phenotype than CRE mice. Macrophage is known to have deficient phagocytosis ability when lacking Hif1-a, which is also confirmed through phagocytosis assay in our lab by FITC marked particles based on melamine resin (P <0.05). We hypothesize the disability of phagocytosis of recipient

resident macrophage renders more DNA debris (also known as DAMP) generated from conditioning, leading to susceptibility of aGVHD, we applied Dnase I to mice from day4 to day14 post-HSCT. The survival of KO mice after injection of Dnase I, with a mean survival of 39.9 days, is better than KO mice with vehicle injection (P <0.05). After Dnase I injection, KO group, compared to 63.5 days mean survival of the control group, there is no statistical difference (P=0.113). In order to look into more mechanisms, CD45 $^{+}$ cells from the liver of KO and CRE mice with GVHD model 21 days post-HSCT were isolated to do single-cell sequencing. KO mice revealed more T cell ratio than CRE mice. GO analysis revealed several abnormalities including regulation of lymphocyte activation.

Conclusions

We build GVHD model on LysM $^{cre/cre}$-Hif1a $^{flox/flox}$ and LysM $^{cre/cre}$ mice, both adopt alloHSCT from wild-type BALB/c mice. After HSCT, both groups are replaced with wild-type neutrophils and circulating monocyte/macrophages, leaving resident macrophages different. KO mice present with exacerbated GVHD phenotype than CRE mice after alloHSCT. This difference could be mitigated by Dnase I injection. Thus, the resident macrophage of the recipient plays a crucial role in aGVHD occurrence, which is partly by regulation of DAMP phagocytosis.

Disclosures No relevant conflicts of interest to declare.

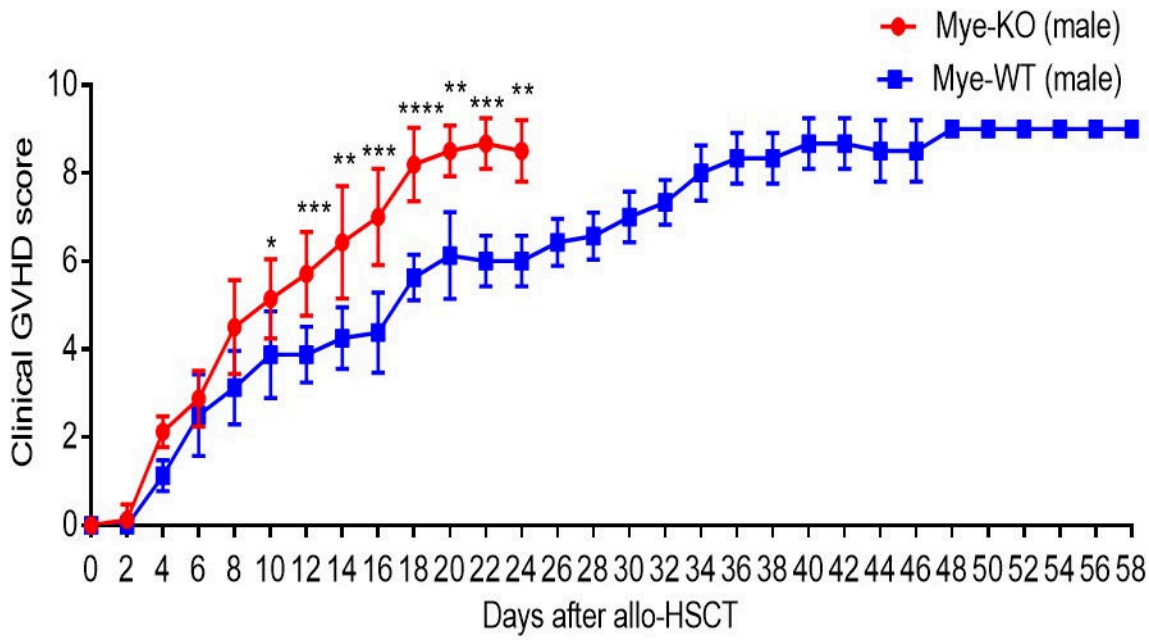

Figure 1:aGVHD score was measured after allogeneic stem cell transplant from wild-type mice of BALB/c, LysMcre/cre-Hif1a flox/flox mice present with a more severe GVHD phenotype than LysMcre/cre mice

Figure 1

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