ELSEVIER

Blood 142 (2023) 4792

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

701.EXPERIMENTAL TRANSPLANTATION: BASIC AND TRANSLATIONAL

Itaconate Inhibits Th1 Differentiation and aGVHD Via Nrf2

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Background:Acute graft-versus-host disease (aGVHD) is one of the major complications after allogeneic hematopoietic stem cell transplantation (allo-HSCT), which is caused by the differentiation of pathogenic T cells and resulted a high mortality. Development of novel therapies is critical for the prevention and treatment of aGVHD.
Aims:The current study aimed to investigate the role of itaconate in the pathogenesis of aGVHD after allo-HSCT.
Methods:To establish murine aGVHD model, lethally irradiated BALB/C (H-2 ^{d}) recipients were injected intravenously with 1×10^{7} bone marrow (BM) cells and 5×10^{6} splenocytes from C57BL/6 or Nrf2 deficiency (H-2 ^{b}) donors. Recipients were intraperitoneally injected with 30mg/kg itaconate once a day 3 days prior to BMT for a period of one week. To explore the potential mechanisms of itaconate in the regulation of aGVHD, we profiled the immune cell responses in splenocytes by flow cytometry 2 weeks post transplantation. To detect a CD4 or CD8 dependent manner, recipients were transplanted with TCD-BMs together with either CD4 or CD8 depleted splenocytes respectively. To elucidate the role of itaconate in Th1 development, naïve CD4 $^{+}$T cells were sorted from WT or Nrf2 KO mice and induced in a Th1 polarization condition for 3 days. IFN-γ production was measured in the supernatant of Th1 cells by ELISA. RNA-Seq was performed to investigate the mechanism of itaconate involved in the regulation of Th1 cell differentiation. Immunofluorescence staining were performed to detect the function of itaconate on Nrf2 expression. The expression of Nrf2 downstream genes were determined by real-time PCR. Total ROS releasing in Th1 cells were detected by a DCFH-DA method.
Results:The survival of receipts administrated with itaconate was significantly prolonged than control mice, companied by a lower aGVHD clinical score and decreased tissue damage. The proportions of CD3 $^{+}$T, CD4 $^{+}$T, CD8 $^{+}$T cells were comparable between recipients treated with PBS or itaconate. T cell activations in recipients treated with itaconate were significantly suppressed compared to those of controls. However, itaconate didn't affect the percentages of Tregs and myeloid subsets, including DCs, macrophages and neutrophils. Interestingly, the population of IFN-γ producing CD4 $^{+}$T, but not CD8 $^{+}$T, was substantially decreased after itaconate treatment. Moreover, productions of IL-6 and IFN-γ in serum were significantly decreased. The protective effect of itaconate were diminished after depletion of donor derived CD4 $^{+}$T cells or IFN-γ. RNA-seq and in vitro studies showed that itaconate could directly inhibit Th1 development by regulating oxidative stress response. Itaconate activated Nrf2 downstream genes, thus suppressed ROS production. Both in vitro and in vivo Th1 differentiation were abrogated after Nrf2 depletion. The protective role of itaconate in aGVHD was also diminished when utilizing Nrf2 deficient mice as donors.
Conclusion:Itaconate significantly inhibited the activation and IFN-γ production of T cells, rather than interrupted the distribution of myeloid subsets, corresponding to mitigated aGVHD development. Itaconate directly suppressed Th1 differentiation and regulated aGVHD by a CD4 dependent manner. Mechanistically, itaconate promoted the expression of Nrf2 and its downstream genes thus suppressed ROS production, leading to a defective Th1 differentiation. Thus, our results revealed a protective role of itaconate in the pathogenesis of aGVHD by regulating Th1 differentiation.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-174152