Ischemia Reperfusion Injury Drives Post-Operative Monocyte Differentiation and Alloreactivity in Orthotopic Liver Transplant Patients

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Abstract

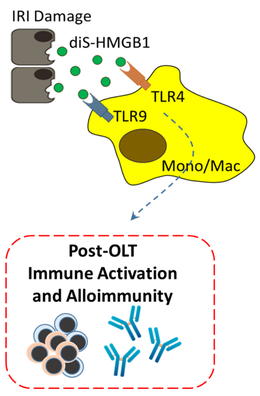
Aim: Ischemia-reperfusion injury (IRI) significantly impacts orthotopic liver transplantation (OLT), affecting graft viability, function, and the risk of chronic rejection. However, the intricate dynamics of innate-adaptive immune interplay, particularly involving circulating monocytes and memory T cells in IRI, remain poorly understood.

Methods: We performed a detailed analysis of circulating monocytes and alloreactive memory T cell subsets in longitudinal PBMCs from 55 OLT recipients (IRI- =28, IRI+ =27) using multicolor flow cytometry. Samples were collected at key time points, before OLT (pre-op), 1-4 months (early), and >6 months (late post-OLT). Activation-induced markers on alloreactive T memory cells were studied after 24h stimulation with donor splenocytes. Additionally, we analyzed the activation of pattern recognition receptors (PRRs) expressed by TLR4- and TLR9-transfected HEK-Blue reporter cell lines stimulated with liver flush perfusate (LF) and disulfide-HMGB1 (DAMP). Results were correlated with IRI status.

Results: Early post-OLT, IRI+ patients showed changes in monocyte subtypes, with increased HLA-DR expression and reduced PD-L1 and CD66a, indicating the generation of proinflammatory sentinels. A positive correlation was observed between disulfide-HMGB1 and classical monocytes (cMO) in IRI+ patients (r=0.43; p=0.04) but not in IRI- patients (r=-0.24; p=0.30). Furthermore, activation of PRRs by LF from IRI+ recipients revealed a significant correlation between TLR4 and HLADR+CD40+iMO (r=0.40; p=0.05), as well as TLR9 and HLADR+CD40+iMO (r=0.47; p=0.01) at the early stage post-LTx. This suggests a strong link between DAMP/PRR activation and circulating monocyte phenotype, along with their ligand CD40L on T cells. Additionally, IRI+ subjects exhibited more alloreactive memory CD4+ T cells, showing subsets with increased activation markers (CD69+; 5.6% vs 3.4%) elevated (IFN-γ 3.6% vs 0.8%) and (IL-17A; 2.2% vs 0.5%) cytokine production, indicating ongoing alloreactivity.

Conclusion: Our findings highlight a pronounced early activation in circulating monocytes and subsequent expansion of memory CD4+ T cell in IRI+ OLT patients. The significant correlation between the monocyte subset HLADR+CD40+iMO and TLR9 suggests a potential mechanism for the switch from innate to adaptive immune responses, leading to enhanced T cell alloreactivity in OLT-IRI.

Background



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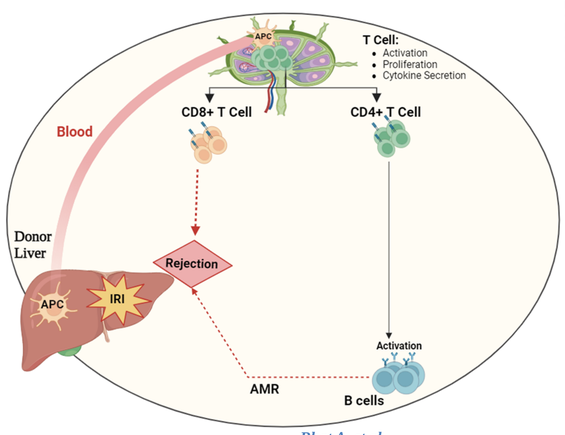

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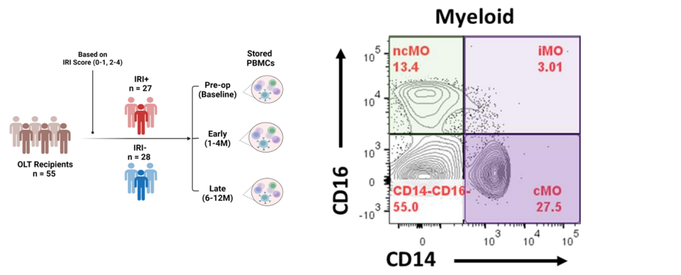
- IRI is a major complication of orthotopic liver transplantation (OLT) that predisposes patients to increased alloimmunity, leading to impairment of allograft function and survival.
- Innate immune cells, such as monocytes and dendritic cells (DCs), play a crucial role in regulating the immune response during IRI. These cells are responsible for maintaining a balance between pro-inflammatory and anti-inflammatory cytokines.
- Understanding this relationship is crucial for developing strategies to mitigate the adverse effects of IRI and improve the outcomes of liver transplantation.
- ***Therefore, we hypothesize that IRI is associated with changes in innate immune response and drives alloreactivity in OLT patients***
- To explore the underlying mechanisms of how IRI affects immune responses, various research methods were employed.

Methods

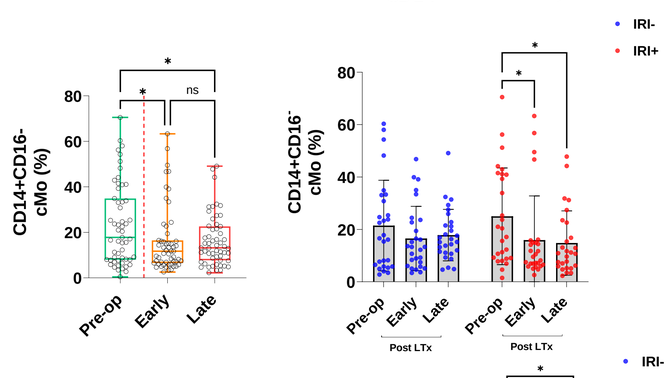
- Phenotype and functional characteristics of circulating monocytes subsets and alloreactive T cell in longitudinal PBMCs from 55 OLT recipients (IRI- =28, IRI+ =27) were assessed by flow cytometry before OLT (pre-op), 1-4 months (early), and >6 months (late) time points post-OLT.
- Activation-induced markers on alloreactive CD4+ T cells were also studied after 24-hour stimulation of recipient PBMCs with donor splenocytes.
- Additionally, we analyzed the activation of pattern recognition receptors (PRRs) expressed by TLR4- and TLR9-transfected HEK-Blue reporter cell lines stimulated with liver flush perfusate (LF) and disulfide-HMGB1 (DAMP). Results were correlated with IRI status.

Results

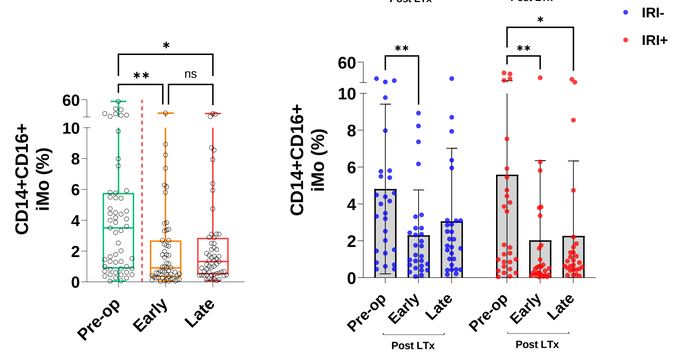
- **Longitudinal Changes in Circulating Monocyte Phenotypes Post-Ischemia-Reperfusion Injury in Orthotopic Liver Transplant Patients**



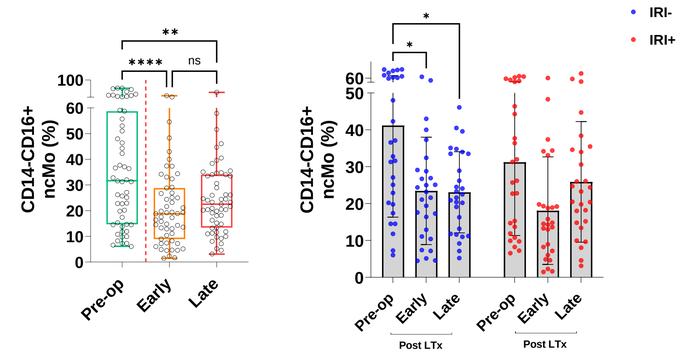
A.



B.

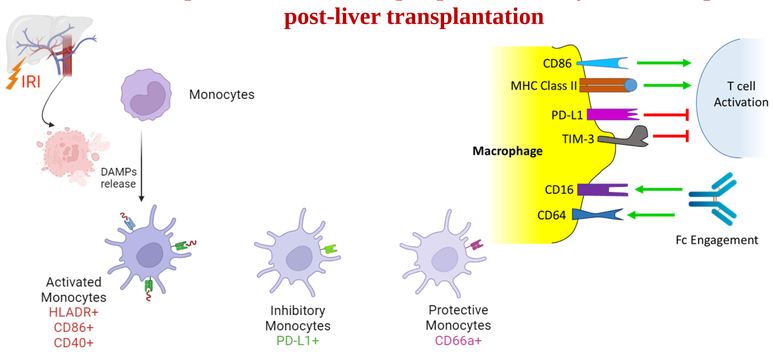


C.



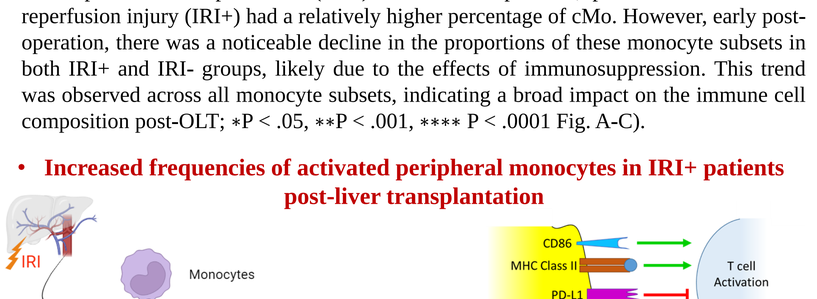
- The data shows a significant decrease in the proportions of classical monocytes (cMo), intermediate monocytes (iMO), and non-classical monocytes (ncMO) following orthotopic liver transplantation (OLT). Before the operation, patients with ischemia-reperfusion injury (IRI+) had a relatively higher percentage of cMo. However, early post-operation, there was a noticeable decline in the proportions of these monocyte subsets in both IRI+ and IRI- groups, likely due to the effects of immunosuppression. This trend was observed across all monocyte subsets, indicating a broad impact on the immune cell composition post-OLT; ∗P < .05, ∗∗P < .001, ∗∗∗∗ P < .0001 Fig. A-C).

- **Increased frequencies of activated peripheral monocytes in IRI+ patients post-liver transplantation**



D. HLADR+ **E.** CD80/86+

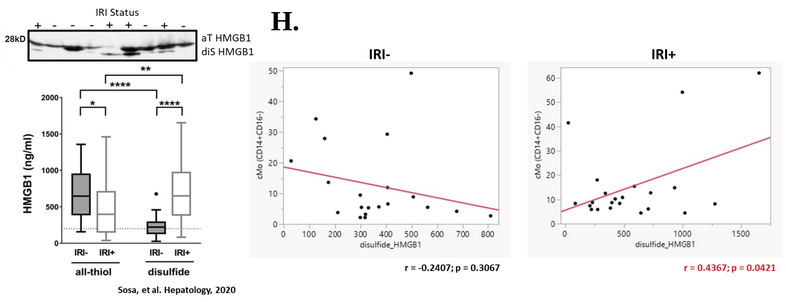
F. PDL1+ **G.** CD66a+



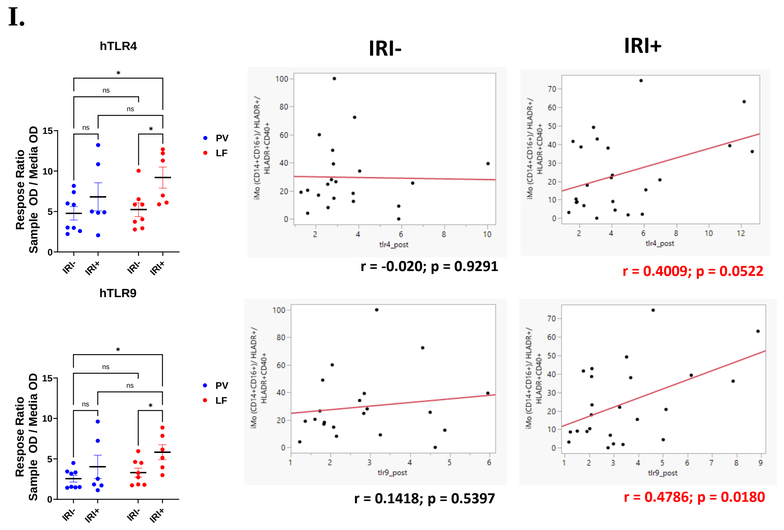
- Classical monocytes in the IRI+ group showed significantly increased frequencies of activation markers HLADR+ and CD86+ post-transplantation compared to the IRI- group (P < 0.01; Fig. D, E). Additionally, these monocytes exhibited higher expression of HLADR+ and CD86+, showing an increasing trend at the late post-OLT stage based on mean fluorescence intensity (MFI).
- cMO in the IRI+ group exhibited significantly lower frequencies of inhibitory markers PD-L1+ and CD66a+ after transplantation compared to the IRI- group (P < 0.01; Fig. F, G) and showed a decreasing trend in the expression of these inhibitory markers at the late post-OLT stage.

- **Correlations Between dis-HMGB1, TLR4/TLR9 Expression, and Monocyte Populations in IRI+ and IRI- Patients**

H.



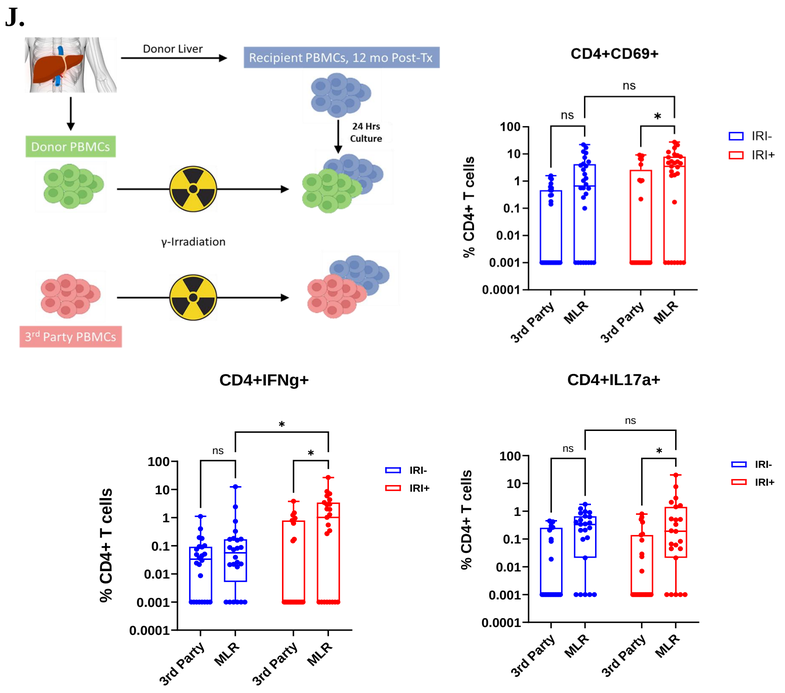
I.



- A significant positive correlation was identified between dis-HMGB1 concentration and the percentage of classical monocytes in IRI+ patients (r = 0.4367; p = 0.0421), while a negative correlation was noted in IRI- patients (r = -0.2407; p = 0.3067) (Fig. H).
- In IRI+ recipients, a positive correlation was also observed between TLR4 expression (r = 0.4009; p = 0.0522) and TLR9 expression (r = 0.4786; p = 0.0180) with the activated monocyte population in vivo (Fig. I).

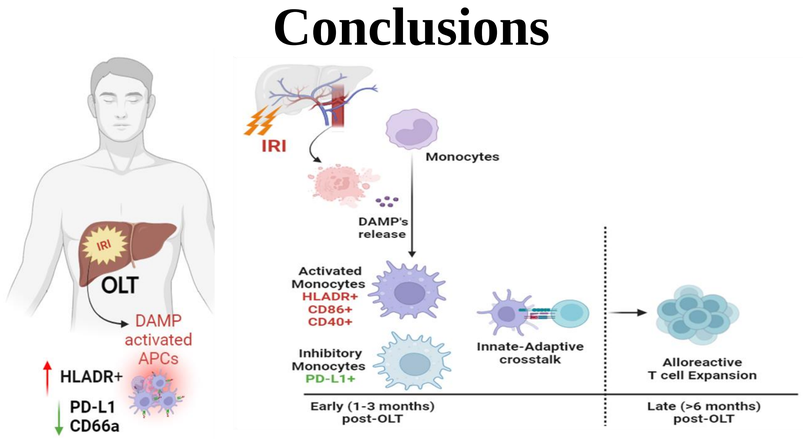
- **Allo-responsive memory CD4+ T cells were more activated and functional in IRI+ recipients upon donor stimulation**

J.



- IRI+ patients have CD4+ T cells, with CD69+ activated and functional IFN-γ and IL-17A-producing alloreactive T cells, which were significantly increased upon donor stimulation at a late time point.(Fig. J).

Conclusions



- ❖ Our results emphasize the complex interplay between innate and adaptive immune responses in the development of alloimmunity following transplantation.
- ❖ In IRI+ patients, we observed significant monocyte activation, marked by an increase in percentages of circulating pro-inflammatory monocyte subsets.
- ❖ These findings suggest that the expansion of alloreactive T cells is closely linked to the activation of circulating monocyte subsets, offering a potential mechanism by which IRI enhances post-liver transplant alloreactivity.

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