MerTK and Axl restrain Kupffer cell inflammatory activation in liver ischemia reperfusion injury

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ABSTRACT

As the dominant tissue resident innate immune cells, Kupffer cells (KCs) play critical roles in liver inflammation and injury. This study took advantage of a KC-specific gene knock-out system, (Clec4F-cre) to determine how Tyro3, Axl, and Mer (TAM) receptor tyrosine kinases (RTK) regulated KCs in liver ischemia reperfusion injury (IRI).

MerTK and Axl are constitutively expressed in tissue resident, but only induced in inflammatory infiltrating, macrophages (MΦs). They both regulate resident MΦ efferocytosis under inflammatory condition. Either MerTK or Axl deficiency in KCs aggravates IRI and increases inflammatory gene expressions in livers. MerTK interreacts with Trem2 to protect livers, as Trem2 blockade diminished the differences between MerTK flox and KO mice in their susceptibilities to liver IRI.

These results demonstrate that efferocytosis receptor MerTK and Axl restrain KC inflammatory activation and protect livers from IRI.

METHODS

Mouse liver partial warm ischemia model.

After a midline laparotomy, mice were injected with heparin (100 mg/kg) and an atraumatic clip was used to interrupt arterial/portal venous blood supply to the cephalad-liver lobes. After 60 min of ischemia, the clip was removed to initiate hepatic reperfusion. Sham controls underwent the same procedure, but without vascular occlusion. Mice were sacrificed after 6h of reperfusion, and liver and serum samples were collected. Parts of liver specimens were fixed in 10% buffered formalin and embedded in paraffin. Liver sections (4mm) were stained with hematoxylin and eosin (HE).

Peritoneal macrophage cultures and Zymosan-induced peritonitis model.

Peritoneal MΦs were collected by i.p. injection of 5 mL DMEM and 1 × 10^6 cells were seeded in 24 well plates. After 2 h incubation, nonadherent cells were rinsed off by PBS. The adherent cells were enriched with macrophages and used for functional assays.

Zymosan was injected i.p. at 10 or 100μg/mouse. Peritoneal lavage were collected at 4h, 24h or 48h post injection.

Efferocytosis assay.

Jurkat cell apoptosis was induced by 200 nM staurosorine for 12 h. After washing with cold PBS, cells were labeled with pHrodoSE. The labeled apoptotic cells were then cocultured with peritoneal macrophages at 2-4:1 ratio for 2 hours. Macrophages were rinsed with PBS to remove unbound Jurkat cells and collected for FACS analysis. Macrophages were identified as CD11b+F4/80+ and TIM-4 was used to differentiate resident vs. infiltrating macrophages.

RESULTS

1.MerTK and Axl Expressions in resident and infiltrating MΦs

To study TAM RTK expressions in different macrophages, we isolated peritoneal resident macrophages in sham mice, infiltrating macrophages from ZIP mice at 4, 24 and 48h post zymosan injection (100ug, i.p.). As shown in Fig.1A, MerTK are expressed in all, but Axl in only 30% of, resident macrophages. Infiltrating macrophages express neither of these molecules at 4h but are induced to express both of them at 48h in a similar pattern as rMΦs. We also analyzed how peritoneal inflammation regulates MerTK expressions in resident and infiltrating MΦs in the ZIP model (10ug). Peritoneal lavages were collected from sham or ZIP mice at 4, 24, and 48h post zymosan injection. Resident and infiltrating MΦs were differentiated by TIM-4 expression. As shown in Fig.1B, MerTK levels in rMΦs reduced at 4h post zymosan injection and recovered at 48h. Meanwhile, MerTK was not expressed in iMΦs at 4h and induced at comparable levels at 48h.

These data demonstrate that MerTK and Axl are constitutively expressed in rMΦs and downregulated during tissue inflammation. The iMΦs do not express these molecules in the acute phase of tissue inflammation but are induced later in the resolution phase.

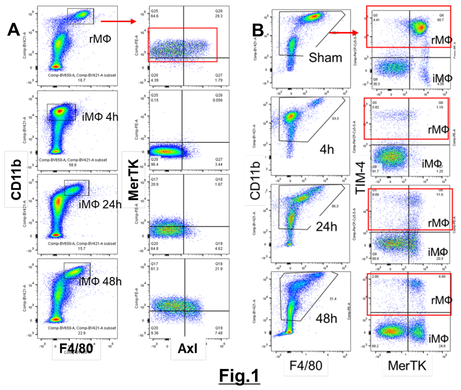

Fig.1

Fig.1 Flowcytometry analysis of MerTK and Axl expression in peritoneal macrophages. (A) Peritoneal lavages were collected from sham or ZIP mice at 4h, 24h and 48h post zymosan injection (100μg, i.p.). Cells were stained with fluorochrome labeled Abs and analyzed by flowcytometry. Representative dot plots were shown, CD45+CD11b+F4/8-+ cells were gated and further measured Axl and MerTK expression. (B) Peritoneal lavages were collected from sham or ZIP mice at 4h, 24h and 48h post zymosan injection (10μg, i.p.). Cells were stained with fluorochrome labeled Abs and analyzed by flowcytometry. Representative dot plots were shown, CD45+CD11b+F4/8-+ cells were gated and further measured MerTK and TIM-4 expression. The rMΦs were TIM-4+ and iMΦs were TIM-4-.

2. MerTK and Axl regulate efferocytosis in resident vs. infiltrating MΦs

MerTK and Axl are major efferocytosis receptors in macrophages. To study whether they differentially regulate resident and infiltrating MΦs under homeostatic and inflammation conditions, we took advantage the ZIP models, in which different types of MΦs were readily accessible. We isolated rMΦs from sham mice of either control (Flox) or MerTK KO or Axl KO (Lyz-Cre) mice and measured their capacities of efferocytosis in vitro by incubating with pHrodoSE-labeled apoptotic Jurkat cells. Our results show that (i) MerTK deficiency reduced by 40%, while Axl deficiency had no impact on, rMΦ efferocytosis under homeostatic condition (Fig.2A, C). (ii) Both MerTK and Axl deficiency reduced rMΦ efferocytosis under inflammatory condition, by 40% or 20% respectively (Fig.2B, C). MerTK deficiency also reduced iMΦ efferocytosis, but only by 20%; while Axl deficiency minimally impacted iMΦ efferocytosis (Fig.2B, C).

These results indicate that MerTK plays a dominant regulatory role in rMΦ efferocytosis under both homeostatic and inflammatory conditions. Axl regulates rMΦ efferocytosis only under inflammatory conditions.

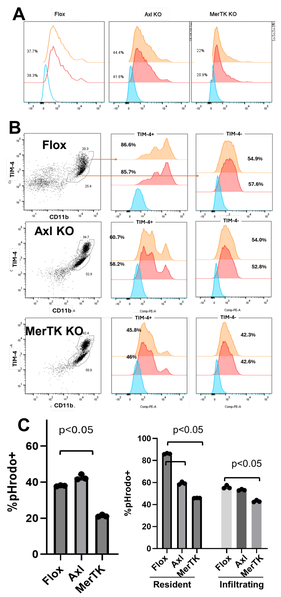

Fig.2

Fig.2 Flowcytometry analysis of macrophages efferocytosis. (A) Histograms of pHrodiSE positive peritoneal resident macrophages (CD11b+F4/80+TIM-4+ cells). (B) Dot plots of peritoneal resident and infiltrating macrophages based on TIM-4 expressions. Histograms of pHrodoSE positive cells in TIM-4+ and TIM-4- macrophages. (C) Average percentages of pHrodoSE positive macrophages were plotted. Representative results n=3

3. MerTK and Axl restrain KC inflammatory activation in liver IRI

To determine whether MerTK and Axl regulate KC functions in liver inflammatory response to IR, we created KC-specific Axl or MerTK deficient mice by crossing Axl/MerTK-floxed with Clec4F-Cre mice. When we compared Axl- or MerTK-floxed (Ctl) with KC-specific Axl or MerTK KO mice in their susceptibilities to liver IR, the KO mice developed significantly more severe IRI, as documented by higher sALT levels, worse liver histopathological pictures and enhanced inflammatory gene inductions (Fig.3). These data indicate that MerTK and Axl restrain KC inflammatory activation in liver IRI.

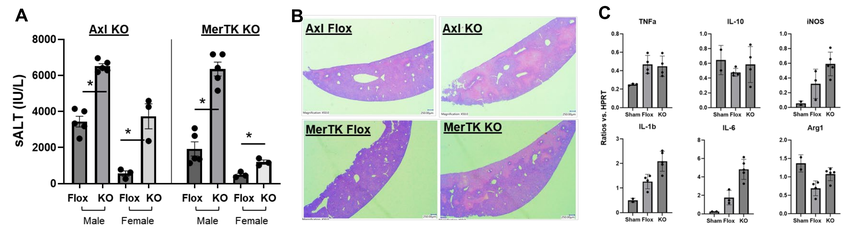

Fig.3 MerTK and Axl regulate KC inflammatory response in liver IRI. (A) Average sALT levels in different groups of mice. Both male and female Axl or MerTK flox or KO (Clec4F-cre) mice were subjected to 60m warm ischemia followed by 6h reperfusion. Average serum ALT levels were plotted. (B) Representative liver histological pictures of Axl or MerTK flox or KO mice were shown (H/E, x50). (C) Liver inflammatory gene expressions were measured by qRT-PCR. Average ratios of target gene/HPRT were plotted.

4. MerTK interacts with Trem2 to protect livers from IRI.

Trem2 (Triggering receptors expressed on myeloid cells) has been identified recently as a critical immune regulatory receptor in macrophages. It can act as an efferocytosis receptor, capable of binding to phospholipids. Blockade of Trem2 signaling aggravates liver IRI in our model (Fig.4). Interestingly, the same anti-Trem2 Ab failed to further increase liver IRI in KC-specific MerTK KO mice, indicating that MerTK is critical in Trem2-mediated immune regulatory mechanism.

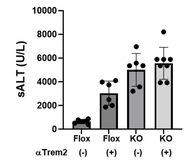

Fig.3 MerTK is critical for the immune regulatory function of Trem2. MerTK flox or KO (Clec4F-cre) mice were treated with either control IgG or anti-Trem2 Ab 1h prior to the onset of liver ischemia. Liver IRI was evaluated at 6h post reperfusion. Average serum ALT levels of different exp. groups were plotted.

CONCLUSIONS

1. MerTK and Axl are expressed constitutively in the resident macrophages, and induced in infiltrating macrophages;
2. MerTK regulates resident macrophage efferocytosis in both homeostatic and inflammatory conditions, while Axl only regulates resident macrophage efferocytosis in inflammatory condition;
3. MerTK and Axl restrain KC inflammatory activation and protect livers from IRI.
4. MerTK is critical for Trem2-mediated immune regulatory mechanism.

ACKNOWLEDGMENT

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