Stimulation of TLR7/8 results in direct IL-1β and IL-18 release in human, suggesting a novel pathway for inflammasome activation

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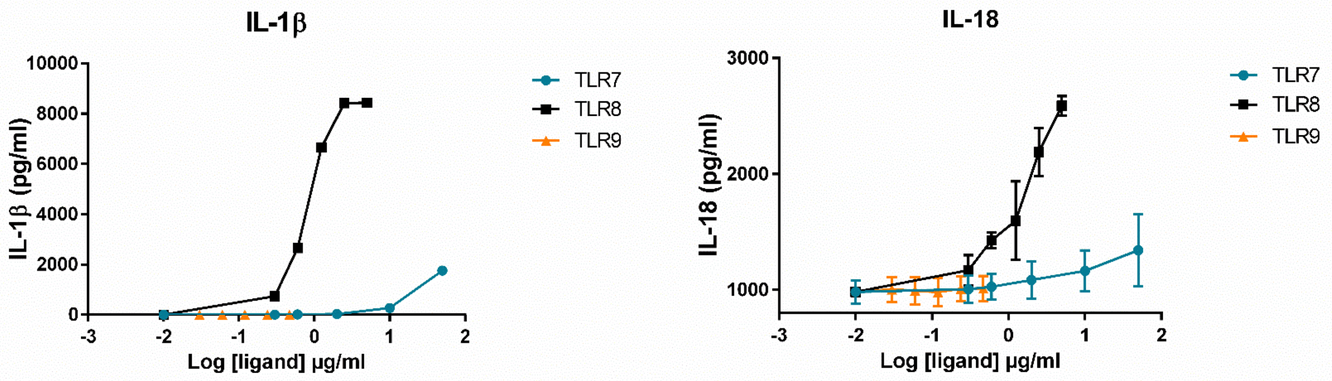
INTRODUCTION & AIM

Toll-like receptors (TLR) play an important role in innate immunity by recognizing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). TLRs also prime inflammasome activity by nuclear factor kappa B (NFκB) mediated gene expression of inflammasome components, pro-interleukin (IL)-1β and pro-IL-18. A second signal is necessary for inflammasome complex formation, resulting in both IL-1β and IL-18 maturation and release. Much is known about the role of TLR4 in inflammasome activation, we evaluate the role of TLRs 7, 8 and 9 in primary human blood cells.

METHODS

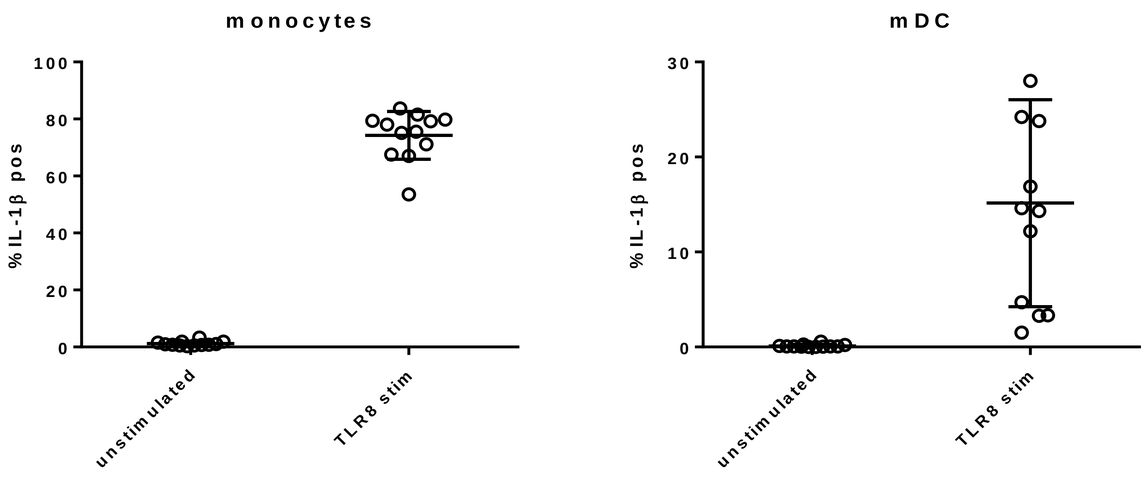
Whole blood or PBMCs were stimulated with LPS (TLR4, 2ng/ml), LPS + ATP (5mM) in the last 30 minutes of incubation, imiquimod (TLR7), ssRNA40/lyovec or a proprietary synthetic nucleic acid (TLR8) or CpG DNA (TLR9) for 3 or 24 hours as indicated. Plasma was assayed for IL-1β and IL-18 release (MSD). Intracellular IL-1β staining was done after 3 hours of stimulation using standard intracellular staining techniques. Monocytes are characterized as $CD14^+$ and mDCs are characterized as $HLA\text{-}DR^+$, CD3, 20 and 56^-, $CD14^-$ and $CD11c^+$. In caspase inhibition experiments, whole blood or PBMCs are pre-incubated with inhibitors for 1 hour prior to stimulation.

TLR7, 8 AND 9 STIMULATION



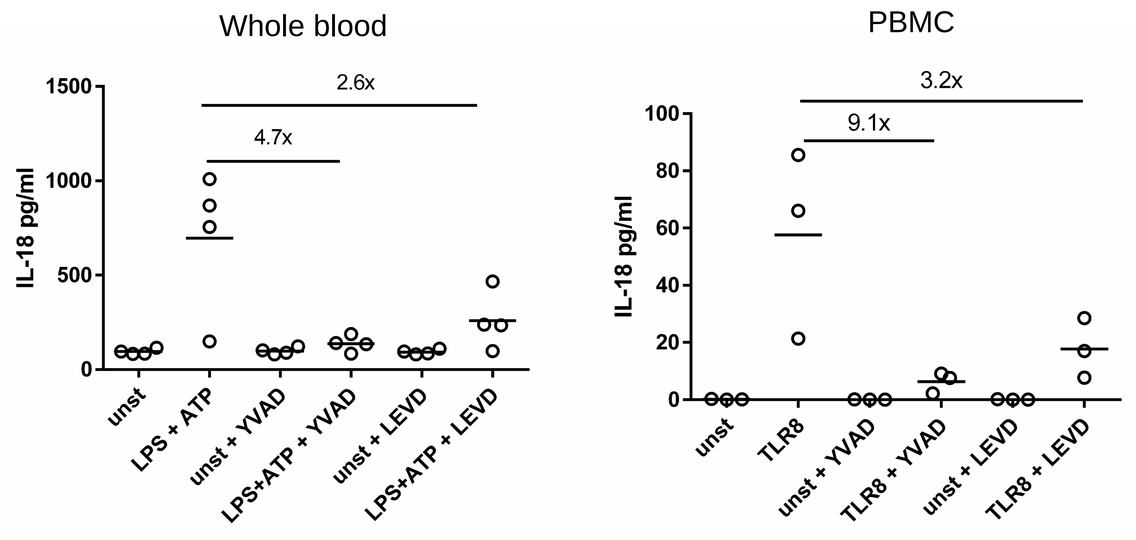
Whole blood was stimulated with TLR7, 8 or 9 agonists for 24 hours. TLR7 and TLR8 stimulation results in IL-1β (n=1) and IL-18 (n=4) release, whereas TLR9 stimulation does not.

INTRACELLULAR IL-1β STAINING



Intracellular IL-1β staining in monocytes and myeloid dendritic cells (mDCs). PBMCs were stimulated with a synthetic TLR8 ligand for 3 hours (n=12). In both experiments monocytes are the main producers of IL-1β, mDC response is highly donor dependent.

CASPASE INHIBITION



Whole blood (n=4) or PBMCs (n=3) were pre-incubated with Ac-YVAD-cmk (caspase-1, -4 and -5) or Ac-LEVD-cho (caspase-4 and -5) inhibitors prior to stimulation. IL-18 release is inhibited by both inhibitors, indicating a role for caspases 4 and/or 5 in inflammasome activation.

CONCLUSIONS

TLR4 stimulation with LPS results in direct IL-1β release, but not IL-18. Surprisingly, stimulation of both TLRs 7 and 8 results in direct IL-1β release and also IL-18. Intracellular IL-1β staining shows that monocytes are the main cell type responsible for IL-1β release after TLR8 stimulation. Caspase inhibition experiments show caspases 4 and or 5 are involved for IL-18 release indicating a role for the non-canonical pathway of inflammasome activation.

FUTURE RESEARCH

To further look into the differences in response between TLR4 and TLR7/8 stimulation regarding the inflammasome, we want to expand on the intracellular staining experiment by looking at different subsets of monocytes. We also want to look into different downstream signalling pathways within the cell. Additionally we would like to characterize the specific inflammasome involved in TLR8 responses.

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