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The differential surface expression of tissue factor between monocyte subsets

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INTRODUCTION

- Sepsis-associated coagulopathy has a high mortality rate and leads to poorer outcomes for individuals with sepsis[1]
- Tissue factor (TF) is the main activator of coagulation *in vivo*. Monocytes are able to express TF on the cell surface and release TF-bearing micro-particles[2]
- Previous work has implicated the monocytic expression of TF in the development of sepsis-associated coagulopathy[2]
- Monocytes are divided into three subsets according to the expression of CD14 and CD16: classical (CD14++, CD16-), intermediate (CD14++, CD16+) and non-classical (CD14+, CD16++)[3]
- Monocyte subsets vary in role and function[3]
- How individual monocyte subsets express TF is unknown

AIMS

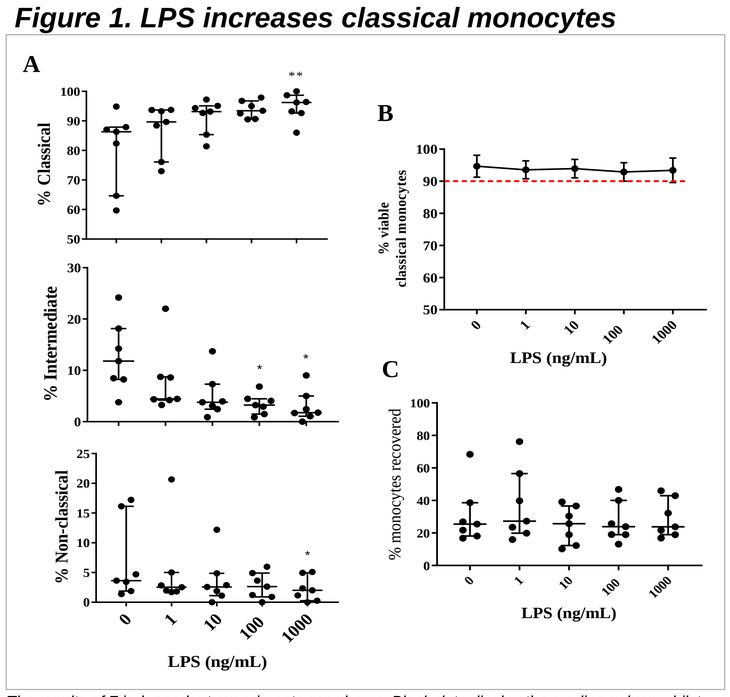
1. Identify how individual monocyte subsets express TF on the cell surface
2. Identify whether monocyte TF plays an active role in sepsis-associated coagulopathy and if activity varies between monocyte subsets
3. Create an *in vitro* model of sepsis using human monocytes and human primary pulmonary microvascular endothelial cells.
4. Investigate the effect of monocyte and endothelial interactions on the expression of monocyte TF and the endothelial fibrinolytic response.

METHODS

- Monocytes and monocyte subsets were isolated from the blood of healthy volunteers using fluorescence-activated cell sorting
- Monocytes were incubated with varying doses of lipopolysaccharide (LPS) for 24 hours. Flow cytometry was used to measure the proportion of monocyte subsets using CD14 and CD16. Cell viability was measured using propidium iodide staining. Fluorescence-minus one controls were used to identify the negative populations
- TF activity was measured using a human TF colorimetric assay that detected activated factor X
- Primary pulmonary microvascular endothelial cells (PMVEC) from a single donor were grown to 90% confluence between passages 2 and 8. Co-cultures of monocytes and PMVEC were incubated for 24 hours. LPS 100 ng/mL was added to PMVEC for 1 hour to stimulate the cells before being washed off. Six co-culture conditions were studied: PMVEC alone, PMVEC stimulated with LPS, PMVEC co-cultured with monocytes, PMVEC stimulated with LPS then co-cultured with monocytes, monocytes alone and monocytes stimulated with LPS 10ng/mL
- Plasminogen activator inhibitor (PAI)-1 was measured using an enzyme-linked immunosorbent assay

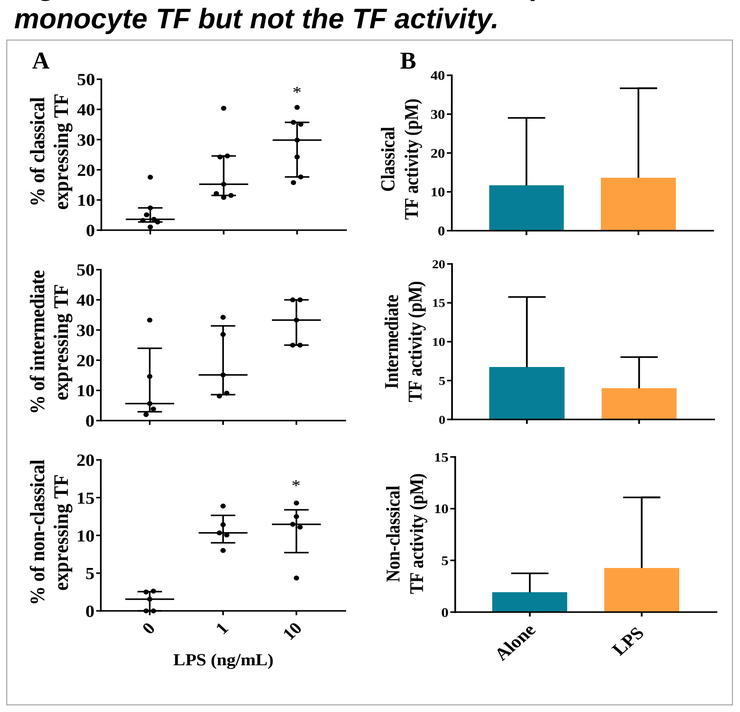
RESULTS

Figure 1. LPS increases classical monocytes



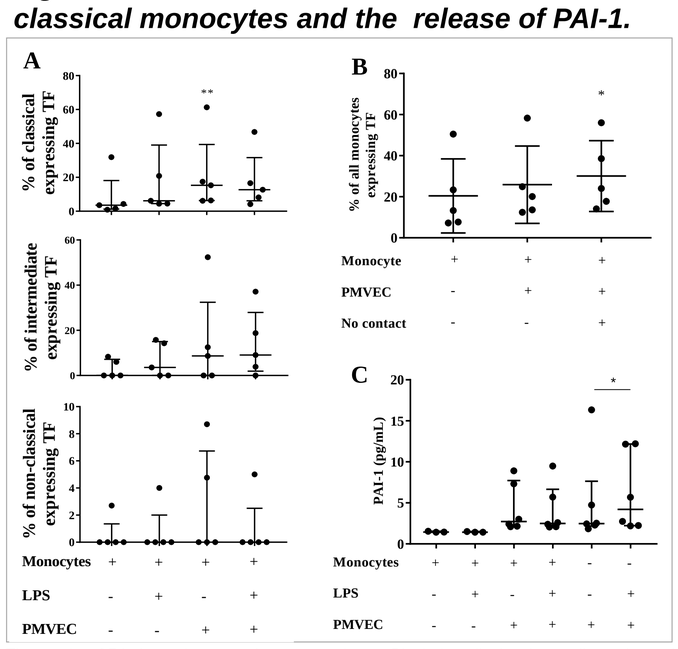
*The results of 7 independent experiments are shown. Black dots display the median values whilst error bars show the interquartile range. (A) Individual monocyte subsets as measured by flow cytometry. (B) Monocyte viability following LPS incubation. The red dashed line denotes 90% viability. (C) Recovery of monocytes following LPS incubation. Significance was calculated using the Friedman test with Dunn's multiple comparisons test used to compare to baseline (no LPS). * = p<0.005, ** = p<0.001*

Figure 2. LPS increases the surface expression of monocyte TF but not the TF activity.



*(A) The TF surface expression of individual monocyte subsets as measured by flow cytometry. The results of 7 independent experiments are shown. Black dots display the median values whilst error bars show the interquartile range. Significance was calculated using the Friedman test with Dunn's multiple comparisons test used to compare to baseline (no LPS). (B) TF activity for each monocyte subset. LPS was used at a dose of 1ng/mL The results of 5 independent experiments are shown. Bars represent median values whilst the error bar shows the upper quartile. Significance was calculated using the Wilcoxon test. * = p<0.005*

Figure 3. Co-culture increases the surface TF on classical monocytes and the release of PAI-1.



*The results of 5 independent experiments are shown. Black dots display the median values whilst error bars show the interquartile range. (A) The TF surface expression of individual monocyte subsets as measured by flow cytometry. ** = p<0.01 for monocytes cultured with unstimulated PMVEC compared to monocytes alone. (B) Repeat of the co-culture model using transwells to prevent cellular contact. * = p<0.05 for monocytes and PMVEC cultured with a transwell compared to monocytes alone. (C) Measurement of PAI-1 following monocyte and PMVEC co-culture. Significance was calculated using the Friedman test with Dunn's multiple comparisons test. * = p<0.005 for LPS-stimulated PMVEC cultured with monocytes compared to LPS-stimulated PMVEC cultured alone.*

- Incubation with LPS led to an increase in the proportion of classical monocytes with a reciprocal decrease in the proportion of intermediate and non-classical monocytes (figure 1, A)
- The change in monocyte subset proportion was not explained by increasing cell death or a drop in yield (figure 1, B & C)

- Monocyte subsets varied in the surface expression of TF Classical and intermediate monocytes showed higher levels than non-classical (figure 2, A)
- LPS induced the monocytic surface expression of TF; classical and intermediate monocytes showed a significant increase in surface TF (figure 2, A)
- There was no variation in the TF activity of monocyte subsets and TF activity was not increased with LPS incubation (figure 2, B)

- Monocyte and PMVEC co-culture lead to an increase in TF surface expression on classical monocytes (figure 3, A); this effect was not dependent on monocyte endothelial contact (figure 2, B)
- The interaction between monocytes and LPS-stimulated PMVEC was associated with an increase in PAI-1 release from the endothelial cells (figure 3, C)

CONCLUSIONS

1. Stimulation with LPS alters the proportion of monocyte subsets
2. Monocyte subsets vary in surface expression of TF both before and post LPS stimulation
3. The LPS-induced increase in monocytic TF surface expression was not accompanied by an increase in TF activity
4. Monocyte and endothelial cells interact to create a pro-thrombotic environment by increasing the monocyte surface expression of TF and increasing PAI-1 release from endothelial cells

Future work investigating sepsis-associated coagulopathy needs to consider the role of individual monocyte subsets and not monocytes as a whole

ACKNOWLEDGEMENTS

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REFERENCES

1. Dhainaut JF, Shorr AF, Macias WL, Kollef MJ, Levi M, Reinhart K, Nelson DR. Dynamic evolution of coagulopathy in the first day of severe sepsis: relationship with mortality and organ failure. Critical care medicine. 2005 Feb 1;33(2):341-8.
2. Levi M, van der Poll T. Coagulation and sepsis. Thrombosis research. 2017 Jan 1;149:38-44.
3. Wong KL, Tai JJ, Wong WC, Han H, Sem X, Yeap WH, Kourilsky P, Wong SC. Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. Blood. 2011 Aug 4;118(5):e16-31.

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