Children with non-critical infections have increased intestinal permeability, plasma endotoxaemia and altered innate immune responses

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Background

- Children with critical illness such as sepsis can have a period of reversible immune dysfunction, where they are more susceptible to infections
- These changes seen in the innate immune system include reduced monocyte HLA-DR expression and TNF- α production
- One of the most potent mediators of this process is lipopolysaccharide (LPS), or endotoxin, a gram-negative cell wall component.
- Elevated circulating levels of LPS are seen not just in gram-negative infections, but also gram-positive infections, trauma, and cardiac arrest
- The presence of this LPS could plausibly be from the gut, which has a large gram-negative load.
- The gut becomes leakier in infections or inflammation, plausibly allowing translocation of this LPS

This study therefore looks at children's intestinal permeability, circulating endotoxin, and some innate immune functions in children with infections and healthy controls.

Methods

Children aged 6-59 months were recruited at the Royal London Hospital.
Cases were children with non-GI infections, documented fever $>38^{\circ}\text{C}$ and CRP $>10\text{mg/L}$
Controls were children not acutely unwell, no fevers and CRP (if measured) $<10\text{mg/L}$.

Participants had a blood test, where their plasma endotoxin was measured, monocyte activation markers were measured (as per **Table 2**), and blood was stimulated for 24h with LPS or the fungal cell wall component Zymosan. Cytokines were measured in blood and following stimulation.

Intestinal permeability was measured with a

lactulose:mannitol (LM) test, where children were given an LM solution and their urine was collected for 2 hours. The concentration of LM in the urine gives an indication of gut permeability.

Results

	Cases	Controls
n	11	19
Age (Months)	25 (6 – 57)	31 (9 – 52)
Male, %	7 (63.6%)	12 (61.3%)
Weight (kg)	12.0 (9.1 – 16.2)	13.4 (6.3 – 27)
CRP	109 (22 – 236)	
White Cell Count	16.3 (6.9 – 34.5)	

The demographics of the children are shown in **Table 1** above. There was no statistical difference between cases and controls.

Plasma Endotoxin and Intestinal Permeability

Cases had higher circulating plasma endotoxin level at a median of 0.24EU/mL compared with 0.02EU/mL in cases ($p=0.028$, **Figure 1a**), as well as increased intestinal permeability (LMR 0.09 v 0.03, $p=0.042$, **Figure 1b**). There was a

loose correlation between the endotoxin and permeability ($r=0.38$, $p=0.08$).

Monocyte activation markers

Monocytes from cases had decreased HLA-DR, and increased CD64 expression (not displayed).

24h stimulation: cytokine production

Following zymosan stimulation, cells from cases produced less TNF- α and IL-1 β (**Figure 2a, b**)

24h stimulation: monocyte markers

Following zymosan stimulation, monocytes from cases displayed decreased HLA-DR compared with controls (**Figure 3a**)

Patients with a higher endotoxin ($>0.1\text{EU/mL}$, 6 cases, 5 controls), had a decreased CD86 and CD64 expression (**Figure 3b**).

Discussion

Both intestinal permeability and plasma endotoxin was increased in cases compared with controls. Given only one of the case's causative organisms was a gram-negative, this increased endotoxin is likely to come from increased bacterial translocation across a more leaky gut wall.

The decreased HLA-DR seen in monocytes in cases represents a decreased ability to respond to future infections, with CD64 being upregulated in infection.

Proteins	Role
HLA-DR	MHC Class II surface receptor, present on antigen presenting cells
CD64	Fc γ RI high-affinity receptor that binds IgG
CD86	T-cell co-stimulator that promotes T-cell activation and survival.

Table 2: Role of proteins examined in this study

Following 24h stimulation with zymosan, cases produced less TNF- α and IL-1 β , and those with a higher endotoxin ($>0.1\text{EU/mL}$ regardless of infection) had reduced CD86 and CD64 expression. Both of these changes have been associated with severity of sepsis, as well as poorer outcomes.

There were therefore differences seen both in children with infection, and those with higher endotoxin levels (regardless of infection), suggesting this endotoxin translocation could be contributing to the poorer outcomes described.