**Methods Supplement**

**Clinical specimens**

Venous blood was taken antenatally. Serum was prepared by centrifugation and specimens were frozen at -80°C until analysis. Case-control pairs were batch assayed to minimize any effect of inter-assay variability.

**Detection anti-CMV IgG, IgM and IgA serology**

Enzyme-linked immunosorbent assays (ELISA) were performed to detect anti-CMV IgG, anti-CMV IgM (Calbiotech, Sprint Valley, CA, USA), and anti-CMV IgA (Diagnostic Automation Inc, CA, USA) in maternal serum samples according to the manufacturers’ instructions. CMV IgG or IgM antibody indices greater than 1.1 were considered positive. CMV IgA antibody indices greater than 1.0 were considered positive.

**Neutrophil isolation**

Briefly, 25ml blood was transferred to 50ml polypropylene tubes with Ca2+- and Mg2+- free Dulbecco PBS (Phosphate buffered saline) (Gibco, Grand Island, NY; total volume 40ml). 6% Dextran sedimentation was performed for 30 minutes, and the resulting leukocyte-rich plasma (LRP) transferred into a new Falcon 50ml tube for centrifugation, re-suspension in 20ml of PBS layered onto 10 ml of Ficoll-Paque Plus, and differential centrifugation. The resulting neutrophil-enriched cell pellet was washed twice in PBS, and red cell lysis performed in 5ml of 1 x RBC lysis buffer for 5min prior to adding with PBS. Two further washes in PBS were performed and the number of cells counted using a haemocytometer.

**Relative quantitative SYBR Green real-time polymerase chain reaction (PCR)**

The sequences of the primer pairs for TLR-2 and TLR-4 are listed as follows:

human TLR-2-sense: 5'-GAATCCTCCAATCAGGCTTCTCT-3'

human TLR-2-antisense: 5'-CCTGAGCTGCCCTTGCA-3'

human TLR-4-sense: 5'-GGCATGCCTGTGCTGAGTT-3'

human TLR-4-antisense: 5'-GGACCGACACACCAATGATG-3'

TLR-2 and TLR-4 mRNA levels were measured with SYBR Green real-time PCR on a Sequence Detection System (ABI Prism 7300, Applied Biosystems). Reactions were prepared in a 96-well MicroAmp optical plate by the addition of a 5µL of cDNA to 20µL of a PCR master mixture consisting of SYBR Green universal PCR master mix and primers. Primers were titrated to check for amplification efficiency. The relative level of the TLR-2 and TLR-4 expression in clinical samples was compared with a calibrator. The calibrator used in all subjects was cDNA generated from a single volunteer at a single time point in the same manner as test samples and aliquot into small samples to avoid freeze-thaw cycles. Quantitation was normalised to an endogenous control 18s rRNA. The calculation formula was shown in the following. For each clinical sample, the TLR-2/TLR-4 C*T* value was normalized as C = C*T* (TLR-2/ TLR-4) - C*T* (18s).[30](#_ENREF_30) This relative expression was measured as: C*T* = C*T* (1) sample- C*T* (1) calibrator. The value used to plot relative TLR-2/TLR-4 expression was calculated using the expression 2- C*T*. [30](#_ENREF_30)

**Flow cytometry** Neutrophil surface TLR-2 and TLR-4 protein expression was examined with flow cytometry (BD FACS Calibur System, Mississauga, ON, Canada). Phycoerythrin (PE)-labelled antibodies against TLR-2 and TLR-4 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and isotype control (PE Mouse IgG2a, k, BD Biosciences Pharmingen, Mississauga, ON, Canada) were used for neutrophil staining. To minimise data variation caused by instrument fluctuation, Quantum PE molecular equivalents of soluble fluorochrome (MESF) kits (Bangs Laboratories, Warrington, PA, USA) were run simultaneously with each sample. TLR-2 and TLR-4 protein expression intensity was determined by a standard calibration curve by plotting the MESF (Y axis) against the peak channel (X axis).

**Cytokine measurements using multiplex immunoassays** Circulating inflammatory (TNF-α, IL-2, IL-6, and IFN-γ), and anti-inflammatory IL-10 cytokine profiles were measured with a multiplexed fluorescent microsphere immunoassay using the Luminex 100 System (Luminex Corporation, Austin, TX, USA). Multiplex bead kit-based assays were performed in duplicate according to the manufacturer’s protocol (LINCO Research, Inc., St. Charles, MO, USA). Thresholds for detection were 0.1pg/ml for all assays.

**Data supplement**

 



