

Supplementary Methods

Sample collection and immunological assays

Mononuclear cells were isolated from whole blood. Samples were collected in glass vials containing 170 IU of lithium heparin per 10 ml of blood (Vacutainer™, Becton Dickinson, Cowley, and Oxford, UK). These samples were diluted with an equal volume of phosphate buffered saline (PBS) (Oxoid, Hampshire, UK) and 10 ml was then carefully layered over 4 ml of Histopaque®1077 (Sigma-Aldrich, UK), in a 15ml conical polypropylene centrifuge tube (Cellstar®, Griener Bio-One, Frickenhausen, Germany). Tubes were centrifuged at 400 *g* for 30 min at 37°C, without braking in an Eppendorf 5702R centrifuge (Eppendorf AG, Hamburg). After centrifugation, supernatants were aspirated to within 0.5 cm of the liquid interface and mononuclear cells aspirated from the interface. Cells were washed twice in 10 ml of Hank's balanced salt solution (HBBS) (Sigma-Aldrich, UK) and centrifuged at 250 *g* for 10 min at room temperature. Cell counts were determined using a Brightline® Haemocytometer (Sigma-Aldrich, UK). PBMCs were resuspended in 4°C freezing media consisting of 90% foetal calf serum (FCS) plus 10% dimethyl sulfoxide (Sigma-Aldrich, UK) at 10⁶ cells/ml. PBMCs were subjected to controlled freezing rates of 1°C per min from 4° to -80°C prior to storage.

Monocytes were purified from whole blood using immunomagnetic separation. Isolated PBMCs were washed with 50 ml of Hank's solution at 350 *g* for 5 min at 23°C. After discarding the supernatant 10 ml of HBBS was added. A small aliquot was used for determination of cell concentration before centrifugation at 350 *g* for 5 min at 23°C. After discarding the supernatant, 80 µl of cold sterile MACS buffer (Miltenyi Biotec, Auburn, CA, USA) was added to 10⁷ cells. For selection of monocytes, 20 µl of CD14+ MicroBeads

(Miltenyi Biotec, Auburn, CA, USA) were added to the cell mixture following incubation in darkness at 4°C for 15 min. Following incubation, cells were washed in 1-2 ml of MACS buffer at 350 *g* for 5 min at 23°C. These cells were then resuspended in 500 µl of MACS buffer and applied to a pre rinsed MACS® Column (Miltenyi Biotec, Auburn, CA, USA) situated in a magnetic field. This column was rinsed 3 times with 500 µl of MACS buffer before removal from the magnetic field. CD14⁺ cells were eluted with 1 ml of MACS buffer and centrifuged at 350 *g* for 5 min at 23°C. Cells were then resuspended in RPMI-1640 with 10% human AB serum, 2mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 10 mmol 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. Cells were then counted and plated at a cell density of 10⁶ cells/ml. Monocytes were isolated at intervals after drainage to assess the time course of responses. During a stimulation period of 24 h at 37°C, monocytes were exposed to differing concentrations of LPS (1, 10 and 100 ng/ml) and cytokine secretion (IL-β, IL-6, IL-10 and IL-1RA) was then assessed by flow cytometry (Beckmann Coulter, FC500 MPL, Fullerton, USA). Purified monocytes at a cell density of 10⁶ cells/ml were plated in duplicate. They were then stimulated with different concentrations of LPS and incubated at 37°C for 24 h (*t*_{stim}). On completion of the stimulation period, cells were centrifuged at 350 *g* for 5 min at 37°C, the supernatants collected and snap-frozen prior to storage at -80°C in a temperature controlled freezer.

To characterize DC subsets, PBMCs were isolated from patients at each sampling time point using the protocol previously described. A 2 tube protocol was utilized to determine plasmacytoid (p) DC and mDC subsets. First, the leukocytes in total PBMC were gated based on viability. A subsequent gate was based on cells demonstrating HLA-DR⁺Lin1⁻. The mDC population was selected from a gate demonstrating CD11c⁺CD1c⁺. The pDC population was

selected from a gate demonstrating CD11c⁻CD1c⁻. This population was then gated on SSC and CD303⁺ to give the pDC population.

Processing of duodenal biopsies

Four to 5 mm thick histologic sections, mounted on gelatinized glass slides, were baked at 65°C for 4 h and dewaxed in xylene. Endogenous peroxidase activity was blocked with 1% hydrogen peroxide in methanol for 15 min. Sections were then processed in a microwave oven twice for 5 min each time at high power for antigen retrieval. Subsequently, the Leica Bond Polymer Refine Detection system (Leica Biosystems, Milton Keynes, UK) was used for occludin detection. After incubation with 1% bovine serum albumin to block nonspecific binding, sections were incubated with rabbit polyclonal anti-occludin antibody (1:20, ZYMED Laboratories) for 1 h, and with biotinylated anti-rabbit immunoglobulin-G for 30 min. Colour was developed with diaminobenzidine (Sigma Fast DAB tablets, D-4293) and counterstained with haematoxylin. Retrieval solution ER1 (Leica Catalogue number AR9961) and retrieval solution ER2 (Leica Catalogue number AR9640) were used for antigen retrieval. For the negative control, the same technique was used on tissue sections substituting the primary antibody with normal rabbit serum. Ten adjacent high power fields (HPFs) at magnification of ×40 from each tissue specimen, extending throughout the biopsy, were selected for evaluation and was performed in a blinded manner. Selection was random, except that all villi had to be perpendicularly sectioned. For each part of the villous a percentage value of occludin expression was obtained by dividing the number of occludin positive enterocytes by the total number of enterocytes.

Morphometric measurements were performed using an interactive digital slide analyser (Hamamatsu NanoZoomer -XR C12000, Hamamatsu Photonics, Shizuoka, Japan). Slides

containing duodenal mucosa were randomly chosen for each patient and examined under oil using an Olympus BH2 microscope. For each patient, 10 randomly chosen mucosal regions were traced using an electromagnetic stylus on a graphic tablet and the morphometric variables of small intestinal total mucosal thickness, villous height, and crypt depth were measured and the average for each was calculated.