SDC 1 - Methods

Animals
Inbred male Lewis (LEW) and Brown Norway (BN) rats weighing 180–200 g were obtained from Charles River WIGA GmbH (Sulzfeld, Germany). All experiments were performed in accordance with the German federal laws regarding the protection of animals. The “Principles of Laboratory Animal Care” (NIH Publication Vol.25, No.28, revised 1996) were followed. The animals were maintained on a 12-h light/dark cycle and provided with commercially available chow (Altromin, Lage, Germany) and tap water *ad libitum*.

Histochemistry and immunohistochemistry
For histochemical and immunohistochemical analysis whole mounts of the intestinal muscularis were prepared as described previously (1,2). Neutrophils and polymorphonuclear granulocytes (myeloperoxidase (MPO) positive cells) were detected using Hanker-Yates reagent (Polysciences Europe GmbH, Eppelheim, Germany) and quantified. ED1- and ED2-positive monocytes and macrophages were assessed using primary antibodies (mouse-anti-rat; 1:500; Serotec GmbH (Düsseldorf, Germany)). For this purpose, muscularis whole mounts were incubated overnight at 4°C in the respective antibody solution followed by three washing periods in phosphate-buffered saline solution (PBS). Incubation with the secondary antibodies (donkey-anti-mouse/Cy3; 1:500; Dianova GmbH, Hamburg, Germany) lasted four hours and was again followed by three washing periods with PBS. In all staining procedures, secondary antibodies without ED1 or ED2 preincubation were used in parallel to ensure specificity. Using a standard streptavidin-biotin method, paraffin-embedded grafts were also stained with anti-rat CD8-antibody (CD8:MCA48GA, Serotec, Düsseldorf, Germany, dilution 1:200). LSAB2 System-HRP (DAKO, Hamburg, Germany) was used as described in the manual.

RNA extraction and quantification of gene expression
Mediator mRNA expression in the small intestinal muscularis was analyzed using SYBR Green two-step real-time reverse transcriptase polymerase chain reaction (RT-PCR) as previously described (2). Total RNA extraction was performed using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the product protocol. DNA contamination was eliminated by using Ambion DNA-free (Ambion Ltd., Huntingdon, Cambridgeshire, UK). Aliquoted RNA (200 ng) was processed for complementary DNA (cDNA) synthesis. Rat primer sequences for 18s/607024; TNF-α/Rn99999017_m1; Interleukin-1β (IL-1β)/Rn00580432_m1; Interleukin-6 (IL-6)/Rn00561420_m1; Interleukin-10 (IL-10)/Rn00563409_m1; CD4/Rn00562286_m1; CD8a/Rn00580577_m1; IFNγ/Rn00594078_m1; MCP-1/Rn00580555_m1; inter-cellular adhesion molecule-1 (ICAM-1)/Rn00564227_m1; Cyclooxygenase-2 (Cox-2)/Rn01483828_m1; iNOS/Rn00561446_m1 and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB)/Rn01399583_m1 (Taqman Gene Expression Assay, Applied Biosystems, Darmstadt, Germany) were chosen as mediators associated with immunologic responses, apoptosis, cell proliferation, inflammation and anti-inflammation used as previously published (3-5). Each mediator-specific amplification was normalized to an endogenous control (18s). The PCR reaction mixture was prepared using Taqman Universal PCR Master Mix (Applied Biosystems, Darmstadt, Germany). PCR conditions on an AbiPrism 7900 HT Fast Real-Time PCR System (Applied Biosystems, Darmstadt, Germany) were as recommended by the manufacturer. Dissociation of the PCR products by a melting curve analysis protocol consistently showed specific single melting peaks for all used primer pairs. Relative quantification was performed using the comparative C\text{\textsubscript{T}} method as described previously by Schmittgen et al. (6).

**Drugs and solutions**

A standard Krebs Ringer buffer solution (KRB) was used as described previously (1). KRB constituents and bethanechol were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany) and from Merck KGaA (Darmstadt, Germany). Phosphate buffered...
saline (PBS) was purchased from Cambrex Bio Science (Verviers, Belgium). Infliximab (Remicade®) was purchased from Essex Pharma GmbH (Munich, Germany).

Reference List


