Supplementary Material & Methods

Mice & Materials

C57BL/6J-*Il10*^{tm1Cgn} (*Il10*KO), B6.129S7-*Rag1*^{tm1Mom}/J (*Rag1*KO), B6.129S2-*Il10rb*^{tm1Agt}/J (*Il10rb*KO) and C57BL/6J were purchased from The Jackson Laboratory (Bar Harbor, Maine, USA). C.B-17 SCID mice and BALB/c mice were ordered from Harlan (Boxmeer, The Netherlands). *LysMcreIl10ra*$^{fl/fl}$*Rag1*KO animals were bred at the St. Jude Children's Research Hospital. Anti-mouseTNF (IgG_{2a} mAb, clone CNTO5048), anti-mouseIL-12/23-p40 (IgG_{2a} mAb, clone CNTO3913) or isotype control mAbs (IgG_{2a} mAb, clone CNTO6601), were a kind gift of Dr. Dave Shealy (Janssen Research & Development) and IL-10Rα antibodies were ordered from Pharmingen (clone 1B1.a).

In situ hybridization and immunohistochemistry

Sections were cut freshly (4.5 μm) and dried overnight at 37ºC. In situ hybridization was performed using the RNAscope Reagent 2.5HD Red system (Advanced Cell Diagnostics, Milan, Italy), using the *Mm-Il10* probe. After development of the FastRed signaling, sections were washed in PBS and then stained using anti-CD3 (Dako, Copenhagen, Denmark) and Goat-anti-Rabbit-AF488 (Invitrogen) and mounted in SlowFade Gold containing DAPI (ThermoFischer Scientific) and imaged using a Leica DM6000B microscope. Images were analyzed using ImageJ software (http://imagej.nih.gov/ij/), and quantified as the number of CD3 negative cells containing at least 2 positive spots as suggested by the manufacturer. Consecutive slides were stained for F4/80 (Clone CI:A3-1, AbD Serotec) and Donkey-anti-Rat-AF488 (Invitrogen) after antigen retrieval with proteinase K solution (Dakocytomation) for 10 min.

Quantitative RT-PCR

mRNA was extracted from mouse colons, CD patient intestinal biopsies, BMDMs or human monocytes/macrophages at the indicated time points with the ISOLATE II RNA Mini Kit (Bioline, QC-Biotech, Alphen ad Rijn, The Netherlands) according to manufacturer’s conditions. Complementary DNA was synthesized from mRNA using Oligo-dT (ThermoFischer Scientific), random hexamer primers (Promega, Madison, USA), RiboLock RNase and RevertAid reverse transcriptase (both ThermoFischer Scientific). Quantitative RT-PCR (qPCR) was performed on a CFX96^{TM} Real-Time System (Bio-Rad Laboratories, Hercules, CA, USA) using Sensifast SYBR green (Bioline) and primers for *Il10, Ifny, Il1b, , Cd206, Nos2, Cd163*, *Osm, CD206, NOS2, IL10* and *IFNG* (see Table below). mRNA levels were normalized against Glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) and gene expression was calculated with the $2^{-\Delta ct}$ method.

Gene		Sequence (5’ to 3’)
Gapdh	forward	TGTGTCCGTCGTGGATCTGA
	reverse	TTGCTGTTGAAGTCGCAGGAG
Il10	forward	TGTCAAATTCATTCATGGCCT
	reverse	ATCGATTTCTCCCCTGTGAA
Cd206	forward	TGTGGTGAGCTGAAAGGTGA
	reverse	CAGGTGTGGGCTCAGGTAGT
Cd163	forward	TGTGCAGTGTCCAAAAGGAG
	reverse	TGTATGCCCTTCCTGGAGTC
Nos2	forward	CACCAAGCTGAACTTGAGCGA
	reverse	GCCCCATAGGAAAAGACTGCA
Il1b	forward	GGTCAAAGGTTTGGAAGCAG
	reverse	TGTGAAATGCCACCTTTTGA
IFNG	forward	TCAGCCATCACTTGGATGAG
	reverse	CGAGATGACTTCGAAAAGCTG
GAPDH	forward	AAGGTGAAGGTCGGAGTCAA
	reverse	AATGAAGGGGTCATTGATGG
IL10	forward	GCTGTCATCCATTTCTTCCC
	reverse	CTCATGGCTTTGTAGATGCCT

Primers for *Ifng, Osm* and *CD206, NOS2* were obtained from Qiagen (Quantitect primers)

Flow cytometry

For analysis of colonic macrophages, colonic tissue was processed as described (16). Antibodies used were obtained from Biolegend: anti-CD45-APC-Cy7 (clone 30-F11), anti-CD11b-PerCP (clone M1/70), αCD64-PE (clone X54-5/7.1), αCD206-AF488 (clone C068C2), from BD Bioscience: anti-Ly6G-AF700 (clone 1A8), and from e-Bioscience: anti-Ly6C-APC (clone HK1.4). Cells were stained with DAPI to discriminate live cells. Colonic macrophages were defined as $DAPI^{-}CD45^{+}CD11b^{+}Ly6G^{-}CD64^{+}$. After indicated incubations non-adherent

cells were aspirated from the BMDM cultures, and adherent BMDMs were removed by washing the plate with ice-cold PBS and scraping, stained with-αCD206-AF488 and, analyzed using a FACS Fortessa (BD) and FlowJo software (Treestar Inc., Ashland, OR). The mean fluorescence intensity (MFI) of CD206 was determined by subtracting the background MFI of unstained cells. Human monocytes were detached with 5 mM EDTA for 15 minutes and stained with αHLA-DR-PE(Clone L243, Biolegend) and αCD206-APC (Clone 19.2, BD Pharmingen). CD206 MFI was determined in the HLA-DR+ population.

Intestinal tissue homogenates

Intestinal tissue was weighed and homogenized in cell lysis buffer (Cell Signaling Technology, Danvers, USA) with protease inhibitors (Roche, Woerden, The Netherlands) using Precellys tissue homogenizer tubes (Bertin Technologies, France) for 4 times 15 min at 5000 rpm at 4°C. Afterwards, samples were spun down for 10 min at 14000 rpm at 4°C and the supernatant was transferred to a clean tube. Protein concentrations were determined by BCA kit (ThermoFischer Scientific, Waltham, USA).

Bone marrow-derived macrophages

Bone marrow was flushed from femur and tibia bones and cultured with RPMI, 10% FCS, penicillin 100 U/ml, streptomycin 100 mg/ml and 10 ng/ml M-CSF (ReproTech, London, UK). After 3-4 days same amount of medium was added to the cultures. After 6-7 days, non-adherent cells were aspirated and the adherent macrophages were incubated with indicated conditions. Anti-TNF (Fab)2 fragments were prepared with the FRAG IT^{TM}Z kit (Genovis, Cambridge, MA, USA) and added in equimolar concentrations as anti-TNF mAbs. Immune complexes were generated by heating the IgG_{2a} isotype control antibody for 1 hour at 63 ºC. BMDMs were stimulated with 100 ng/ml LPS (Sigma-Aldrich, Zwijndrecht, The Netherlands) and 20 ng/ml IFN-y (PeproTech), IgG_{2a} isotype control antibody (10 µg ml), IgG_{2a} immune complex (10 µg ml), anti-TNF mAbs (IgG_{2a}, 10 µg/ml) or anti-TNF Fab (7,2 µg/ml), and in some conditions with the anti-IL-10Rα blocking antibody (10 µg/ml).

Human monocytes

Peripheral blood mononuclear cells (PBMCs) from healthy volunteers were isolated by Ficoll Paque density-gradient centrifugation. After washing, monocytes were isolated by Percoll density-gradient centrifugation. Macrophage differentiation was induced by culturing monocytes with IFN-γ (50 ng/mL) and LPS (100 ng/ml) in RPMI supplemented with 10% heat-inactivated FCS. The full monoclonal anti-TNF adalimumab (Humira®, AbbVie, Wavre, Belgium), anti-TNF Tab fragment certolizumab (Cimzia®, UCB Pharma, Breda, The

Netherlands) in combination with anti-IL-10 (mouse IgG_{2B} clone 23738, R&D systems, Minneapolis, USA) or 10 µg/ml isotype control (mouse IgG_{2B} clone 20116, R&D systems) were all added in a concentration of 10 µg/ml.

ELISA

Cell culture supernatants or intestinal homogenates were analyzed for the presence of IL-10 or TNF by ELISA (all R&D Systems) according to manufacturer's instructions.

Western blot

Samples were run on 10% SDS-PAGE gels under reducing conditions and transferred to nitrocellulose membranes (GE Health Care, Zeist, The Netherlands). Membranes were blocked by incubation in 5% non-fat milk (Nutricia, Wageningen, The Netherlands) in TBST (TBS + 0.1% Tween-20) for 2 hours at room temperature (RT) and subsequently incubated with pSTAT3 (1:500, Clone D3A7, Cell Signaling Technology, Danvers, MA, USA) or β-actin (1:100000, clone AB1978, Sigma, Deisenhofen, Germany) antibodies in 2% milk/TBST overnight at 4˚C. After incubation membranes were washed 3 times with TBST, incubated with HRP conjugated secondary antibodies (1:2000, Dako) in 2% milk/PBST for 2 hours at RT. Expression was detected by Lumilight Plus (Roche). Afterwards blots were stripped in stripping buffer (ThermoFischer Scientific) for 10 min at RT and incubated with STAT3 antibody (1:1000, clone 124H6, Cell Signaling Technology).

Intestinal biopsies of CD patients

IL10, *IFNG, CD206* and *NOS2* mRNA levels and subsequent *IL10*/*IFNG* and *CD206*/*NOS2* ratios were determined in all biopsies and averaged from biopsies before or after treatment.