**Title: Anti-commensal IgG drives intestinal inflammation and type 17 immunity in ulcerative colitis**

**Authors**

Tomas Castro-Dopico1, Thomas W. Dennison1, John R. Ferdinand1, Rebeccah J. Mathews1, Aaron Fleming1, Dean Clift2, Benjamin J. Stewart1, Chenzhi Jing1, Konstantina Strongili3, Larisa I. Labzin2, Edward J.M. Monk1, Kourosh Saeb-Parsy4, Clare E. Bryant5, Simon Clare6, Miles Parkes3 and Menna R. Clatworthy1\*

**Affiliations**

1Molecular Immunity Unit, University of Cambridge Department of Medicine, Cambridge CB2 0QH, UK.

2Medical Research Council, Laboratory of Molecular Biology, Cambridge CB2 0QH, UK.

3Division of Gastroenterology, Cambridge Universities NHS Foundation Trust, Cambridge CB2 0QQ, UK.

4University of Cambridge Department of Surgery, Cambridge CB2 0QQ, UK.

5Department of Veterinary Medicine, University of Cambridge, Cambridge CB3 0ES, UK.

6Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton CB10 1SA, UK.

\*Lead contact: mrc38@cam.ac.uk

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**Summary**

Inflammatory bowel disease is a chronic, relapsing condition with two subtypes, Crohn’s disease and ulcerative colitis (UC). Genome wide association studies in UC implicate a variant in *FCGR2A* that alters the binding affinity of the antibody receptor it encodes, FcγRIIA, for IgG. Here we aimed to understand the mechanisms whereby changes in FcγRIIA affinity would affect inflammation in an IgA-dominated organ. We found a profound induction of anti-commensal IgG and a concomitant increase in activating FcγR signaling in the colonic mucosa of UC patients. Commensal-IgG immune complexes engaged gut-resident FcγR-expressing macrophages, inducing NLRP3- and reactive oxygen species-dependent production of interleukin (IL)-1β and neutrophil-recruiting chemokines. These responses were modulated by the FCGR2A genotype.  In vivo manipulation of macrophage FcγR signal strength in a mouse model of UC determined the magnitude of intestinal inflammation and IL-1β-dependent type 17 immunity. The identification of an important contribution of IgG-FcγR dependent inflammation to UC has therapeutic implications.

**Introduction**

Inflammatory bowel disease (IBD) is a chronic, relapsing condition with two main clinicopathological subtypes, Crohn’s disease (CD) and ulcerative colitis (UC) (Kaser et al., 2010). Susceptibility to IBD is driven by a genetic predisposition to aberrant mucosal responses to commensals (Jostins et al., 2012; McGovern et al., 2015; Neurath, 2014) and characterized by the inappropriate pro­duction of a number of pro-inflammatory cytokines and chemokines (Neurath, 2014). Genome wide association studies (GWAS) have provided critical insights into disease pathogenesis and implicate a variant in *FCGR2A* that alters the binding affinity of the antibody receptor it encodes (Jostins et al., 2012). Fc gamma receptors (FcγRs) bind to the Fc portion of immunoglobulin (Ig)G, are expressed by many immune cells, including macrophages, and mediate the cellular effector functions of IgG antibodies. These cell surface glycoproteins include activating receptors (in humans FcγRIIA, IIIA and IIIB), and a single inhibitory receptor FcγRIIB (Nimmerjahn and Ravetch, 2008; Smith and Clatworthy, 2010). The extent to which IgG immune complexes (IC) activate immune cells is dependent on the relative engagement of activating or inhibitory FcγRs (the A:I ratio).

Genetic variation in FcγRs can alter the A:I ratio and influence susceptibility to a number of autoimmune diseases (Smith and Clatworthy, 2010). A single nucleotide polymorphism (SNP) in *FCGR2A* (rs1801274) leading to an amino acid substitution (histidine to arginine at position 131) results in a lower binding affinity for IgG, reducing the A:I ratio (Willcocks et al., 2009). FcγRIIA-R131 is protective in UC (Jostins et al., 2012), suggesting that IgG may play a pathogenic role in intestinal inflammation. Although *in vitro* studies have identified a generic increase in the production of a number of pro-inflammatory cytokines by immune cells in response to IgG (Uo et al., 2013), there is limited insight into the cellular pathways underpinning this genetic association. Furthermore, IgG antibodies are considered less important in intestinal immunity due to the dominance of IgA at mucosal surfaces (Fagarasan, 2008).

We found a profound induction of anti-commensal IgG and of activating FcγR signaling in the colonic mucosa in UC patients. The resulting commensal-IgG immune complexes engaged gut-resident FcγR-expressing macrophages, inducing NLRP3- and reactive oxygen species (ROS)-dependent production of IL-1β and neutrophil-recruiting chemokines, and this was modulated by FCGR2A genotype. In a murine model of intestinal inflammation, manipulation of macrophage FcγR signal strength determined the magnitude of intestinal inflammation and of IL-1β-dependent induction of type 17 immunity in vivo. Our findings provide insight into the mechanisms mediating IgG-FcγR associated inflammation in UC, which may present therapeutic targets for the treatment of UC.

**Results**

**Anti-commensal IgG is associated with the magnitude of intestinal inflammation**

We first sought to interrogate if there was an increase in local IgG within the gastrointestinal tract during intestinal inflammation, in line with previous reports (Baklien and Brandtzaeg, 1975; Macpherson et al., 1996; Shafir et al., 1986; Uo et al., 2013). Analysis of published transcriptomic data of intestinal biopsies from a cohort of patients with clinically active UC demonstrated a significant enrichment of IgM and IgG heavy chain transcripts within inflamed mucosa (Fig. 1A, S1A). Indeed, upregulation of an *IGH* signature (a cumulative measure of *IGH* gene expression) was specifically associated with diseased tissue in UC compared with non-diseased UC and healthy control colonic tissue (Fig. S1B), implicating humoral responses in disease.Consistent with an increase in local commensal-specific IgG, we observed a significantly higher proportion of luminal commensals bound by IgG in UC stool samples compared with household controls, in contrast to IgA-bound microbes (Fig. 1B, Table S1). Notably, samples with higher levels of IgG-bound commensals were found in patients with the highest disease severity scores (Fig. 1C).

To probe this phenomenon further, we used dextran sodium sulphate (DSS), a colitogen that leads to osmotic epithelial damage, to induce barrier breach-associated inflammation. Exposure of mice to two repeated cycles of DSS (referred to here as chronic DSS (cDSS)) induced an upregulation of IgG transcripts within the colonic mucosa (Fig. S1C), an increase in luminal IgG (Fig. 1D), and a modest increase in IgA, mirroring observations in human UC. Indeed, a single acute 6-day course of 2% DSS (referred to here as acute DSS (aDSS)) was sufficient to induce an increase in *de novo* circulating anti-commensal and anti-flagellin IgG, consistent with previous reports (Kobayashi et al., 2009) (Fig. S1D). We also observed a significant increase in luminal microbes bound by IgG at day 7 and day 21 post-aDSS exposure compared with day 0, and the addition of paired DSS serum further increased bacterial binding by IgG (Fig. 1E), with no significant increase in IgA-bound bacteria (Fig. S1E). The number of luminal IgG-bound microbes correlated with colon length and colonic neutrophil infiltration (Fig. 1F), measures of disease severity. Indeed, the level of IgG-opsonized microbes better reflected colitis severity than that of IgA opsonization (Fig. S1F), implicating anti-commensal IgG as a determinant of the intestinal inflammatory response. Circulating anti-commensal IgG titers in serum correlated with colonic neutrophil infiltration at day 21 following aDSS exposure (Fig. S1G). This serum IgG response was not specific to a single commensal or pathobiont, but exhibited broad microbial-specificity, including the probiotic *Lactobacillus reuteri* and the pathobiont *Proteus mirabilis* (Fig. 1G), with *L. reuteri* IgG binding correlating most strongly with disease activity of those species tested (Fig. S1H). Consistent with an expansion of local IgG production, we observed an increase in IgG-expressing cells within the inflamed submucosa of the colon of cDSS-treated mice (Fig. 1H), with B cells representing the major lymphocyte population in this setting (Fig. S1I). By day 21 post-aDSS exposure, there was a pronounced expansion of IgG+ CD19+ B cells within the inflamed colonic lamina propria (LP) (Fig. 1I, S1J) and colon-draining mesenteric lymph nodes (MLN) (Fig. 1J, S1M). We also observed an increase in CD138+ plasma cells within the inflamed colon (Fig. S1K) and MLN (Fig. S1N), with an increase in the absolute numbers of IgG-expressing plasma cell at both sites (Fig. S1L, O), indicating that although the transudation of systemic IgG may make a substantial contribution to the luminal commensal-reactive IgG, there is also *de novo* IgG generation locally within the GI tract. Together, these data suggest that in UC there is an increase in intestinal commensal-specific IgG, positioned to activate FcγR-expressing mucosal immune cells and that DSS colitis provides is a reasonable model to interrogate the effects of local IgG in intestinal inflammation.

**Activating FcγR signaling in intestinal inflammation**

To address the role of FcγR signaling in intestinal inflammation, we analyzed FcγR mRNA expression in mucosal biopsies in published cohorts of UC patients. Activating FcγR gene transcripts, including *FCGR2A* and *FCGR3A/B*, were among the most differentially expressed genes in inflamed UC biopsies compared to UC in remission, non-inflamed UC biopsies and healthy control biopsies (log2 fold change (FC) > 2; Fig. 2A, B). In contrast, a more modest induction of the inhibitory receptor, *FCGR2B*, was observed(Fig. 2B), resulting in an increase in the mucosal FcγR A:I ratio (Fig. S2A). UC-associated enrichment was specific for FcγRs and was not observed for other Fc receptors (Fig. S2B) and *FCGR* enrichment was confirmed in a further independent UC dataset (Fig. S2C). Furthermore, in mucosal UC biopsies taken prior to infliximab intervention (Table S2), elevated *FCGR2A* expression was associated with treatment-refractory disease (Fig. 2C) and was predictive of subsequent resistance to tumor necrosis factor (TNF)alpha blockade (Arijs et al., 2009a; Arijs et al., 2009b) (Fig. 2D).

The functional effect of a higher A:I ratio at a cellular level is to reduce the activation threshold, increasing the likelihood of activation upon IgG IC encounter. In keeping with this, we found an enrichment of genes associated with FcγR-mediated phagocytosis in UC biopsies compared with controls (Fig. 2E), a unique pathway distinct from TLR signaling genes (Fig. S2D). Analysis of colonic FcγR expression in cDSS-treated mice, using both a published transcriptomics dataset and confirmatory qPCR, demonstrated a similar increase in FcγR transcripts (Fig. S2E), and enrichment of FcγR activation pathway genes (Fig. 2E). In contrast, a consistent enrichment of FcγR genes or FcγR signaling pathways in *Citrobacter rodentium* infection was not observed (Fig. S2F), suggesting our observations may be specific to DSS-induced colitis.

A number of immune cell subsets express FcγRs, including macrophages, dendritic cells (DCs) and neutrophils. During aDSS-induced inflammation, when compared to other colonic-resident immune cells (as identified in Fig. S2G), intestinal macrophages (CD11b+ CX3CR1+ Ly6Clo MHC-II+ F4/80+) expressed the highest levels of both activating and inhibitory FcγRs (Fig. 2F). In particular, FcγRIII (the murine orthologue of FcγRIIA) was highly expressed at both transcript and protein level (Fig. 2F, G). To investigate FcγR expression in the human intestine, we made use of a recently published single cell RNAseq dataset generated from human colon. Immune cell subsets were identified through canonical marker gene expression and alignment with human PBMC single cell transcriptomic data (Fig. S2H). Analysis of colonic single cell transcriptomes indicated that *FCGR2A* and *FCER1G* were highly specific to the mononuclear phagocyte (MNP) cluster (Fig. 2H), transcriptionally similar to circulating CD14+ classical monocytes (Fig. S2H). Indeed, FcγRIIA was confirmed as the dominant FcγR expressed by CD14+ CX3CR1+ macrophages flow-sorted from the human intestine (Fig. 2I, S2I, J). The increase in activating *FCGR2A* transcripts observed in colonic biopsies in UC might be due to an increase in the number of FcγR-expressing macrophages during inflammation, or the upregulation of activating FcγR expression on resident macrophages. The adult intestinal macrophage pool is largely derived from circulating monocytes in homeostasis and inflammation (Bain et al., 2014). Analysis of the CD11b+ CX3CR1+ “monocyte waterfall” (Tamoutounour et al., 2012) demonstrated an influx of colonic monocytes and an increase in newly-differentiated inflammatory Ly6C+ MHC-IIint macrophages and mature Ly6Clo MHC-IIhi macrophages at day 7 post-aDSS (Fig. 2J). Furthermore, activating FcγR expression was augmented on these cells, increasing their A:I ratio (Fig. 2K), rendering them more susceptible to IgG-mediated activation. Together, these data show that the intestinal MNP system is primed to respond to the emergence of local IgG following the onset of intestinal inflammation.

**Mucosal FcγR expression correlates with IL-1β and CXCL8**

To understand the specific molecular pathways that mediate FcγR-induced intestinal inflammation in UC, we first sought to identify common inflammatory networks present in colonic biopsies across multiple UC cohorts, and then to interrogate correlations with *FCGR2A*. Analysis of significantly enriched cytokines and chemokines (adj. *P* val. < 0.05) within inflamed UC mucosal biopsies revealed that *IL1B* and neutrophil-recruiting chemokines, including *CXCL1* and *CXCL8*, were amongst the most significantly upregulated genes compared to controls (Fig. 3A, S3A). Of these genes, *IL1B* and *CXCL8* correlated most strongly with *FCGR2A* (Fig. 3B, C, Fig. S3B). We also observed a correlation between these genes and *IGHG1* expression (Fig. S3C). To further examine gene expression associations in an unbiased manner, we performed hierarchical clustering of all chemokine and cytokine gene transcripts and *FCGR2A* within these same cohorts. Strikingly, this placed *IL1B* in a cluster with, and adjacent to *FCGR2A* (Fig. 3D, Fig. S3D). In cDSS colitis, as in UC biopsies, *Il1b* was the most highly induced cytokine gene within the inflamed colon, as assessed by transcriptomic analysis, and displayed higher total expression levels relative to other inflammatory mediators by qPCR, suggesting a dominant role for this cytokine in colitis (Fig. S3E). Furthermore, its expression closely correlated with *Fcgr3* transcript expression within whole colonic tissue by qPCR (Fig. 3E), as did *Cxcl1* and *Cxcl2* (murine neutrophil-recruiting chemokines, Fig. 3F). Together these data demonstrate that the expression of FcγRIIA and IL-1β are closely associated during colitis and raise the possibility that anti-commensal IgG may contribute to intestinal inflammation via FcγR-dependent induction of IL-1β, a Th17-polarising cytokine (Chung et al., 2009; Shaw et al., 2012), and neutrophil-recruiting chemokines.

To determine whether IgG and activating FcγR signaling and these key cytokines and chemokines might be causatively linked, we isolated human gut LP mononuclear cells (LPMCs) and stimulated them with IgG IC *ex vivo* to directly cross-link FcγRs. This resulted in a significant induction of *IL1B* and *CXCL8* (Fig. 3G). Similarly, *ex vivo* stimulation of murine LPMCs from aDSS-treated mice with IgG IC resulted in an increase in IL-1β, CXCL1 and CXCL2 production (Fig. 3H). *In vivo*, passive transfer of anti-commensal IgG further enhanced *Il1b*, *Cxcl1* and *Cxcl2* transcript levels within the colon following 7-day aDSS (Fig. S3F). Together, these data support the hypothesis that commensal IgG directly drives intestinal inflammation via induction of pro-inflammatory cytokines and chemokines on resident intestinal immune cells. Intracellular cytokine staining of murine LPMCs demonstrated that CD11b+ CX3CR1+ cells (population A), comprising intestinal MNPs, were the dominant source of IL-1β during colitis, with a minor contribution from neutrophils (Fig. 3I), consistent with our analysis of human colonic single cell RNAseq data, showing that *IL1B* expression is specific to the MNP cluster (Fig. 2H). Indeed, flow-sorted murine colonic macrophages stimulated with IgG IC *ex vivo* demonstrated substantial induction of IL-1β, CXCL1 and CXCL2 expression (Fig. S3G). Finally, we immunized and boosted C57BL/6 mice to generate high titers of circulating anti-flagellin IgG (Fig. S3H). Transfer of this anti-flagellin-enriched serum IgG into naïve IgG-deficient *Rag2-/-* mice prior to aDSS significantly augmented pro-IL-1β expression by colonic CX3CR1+ MNP subsets, particularly Ly6Chi monocytes and Ly6C+ MHC-IIint inflammatory macrophages (Fig. 3J), and was associated with increased weight loss (Fig. 3K, S3I). In contrast, no difference in colonic neutrophil pro-IL-1β expression was observed, suggesting FcγR signaling drives pro-IL-1β expression by colonic MNPs and is sufficient to induce inflammation in the absence of adaptive immune cells.

**IgG-induced IL-1β production by human macrophages**

We next sought to further interrogate the role of FcγR signaling in intestinal macrophage function. A cell-intrinsic effect of IgG IC on macrophage cytokine and chemokine production was evident as FcγR cross-linking on flow-sorted murine CX3CR1+ Ly6Clo MHC-IIhi colonic macrophages induced widespread changes in gene expression (Fig. S4A), including 264 genes that were significantly differentially expressed in UC biopsies (Fig. 4A). IC-induced genes were enriched in UC-relevant immune pathways, such as neutrophil chemotaxis (Fig. S4B), and included key UC-associated cytokines such as *Il1b*, *Il23a*, and the recently characterized *Osm* (West et al., 2017) (Fig. 4A, Fig. S4C). Given our previous analyses demonstrating the predominance of *IL1B* expression in inflamed UC biopsies, that its expression is closely linked to *FCGR2A*, and that *IL1B* is induced in lamina propria MNP following FcγR engagement (Figure 3), we sought to determine the molecular pathway linking FcγR cross-linking and IL-1β production. FcγR signaling resulted in the induction of genes involved in NLRP3 inflammasome priming and activation in murine colonic macrophages, including *Nlrp3* (Fig. 4B, Fig. S4D), suggesting that IgG-associated IL-1β production by intestinal macrophages may be dependent on the NLRP3 inflammasome. Inflammasome assembly is a two-step process; ‘Signal 1’ is required for NLRP3 transcription, and ‘Signal 2’, including stimuli such as ATP and reactive oxygen species (ROS), for the generation of a multimeric complex containing NLRP3, ASC and pro-caspase-1 (Schroder and Tschopp, 2010). Priming of murine bone marrow-derived macrophages (BMDMs) with IgG IC, followed by ATP, resulted in IL-1β secretion in wild type (WT) but not *Nlrp3*-deficient macrophages (Fig. 4C). This induction was greater than either model antigen (Ova) or anti-Ova immune serum stimulation alone (Fig. S4E). IgG IC stimulation of human monocyte-derived macrophages (MDMs) in the presence of a fecal commensal suspension or LPS was sufficient to induce mature IL-1β secretion, an effect almost entirely abrogated by blockade of the UC-associated receptor, FcγRIIA (Fig. 4D, S4F). Both IgG-IC and fecal commensals were effective in inducing *IL1B* transcript and pro-IL-1β protein expression (Fig. 4E). IgG IC alone was insufficient to generate cleaved IL-1β, and although stimulation with fecal commensals resulted in the production of some mature IL-1β, only the combined stimulation of IgG IC and fecal commensals was effective in driving substantial cleavage of pro-IL-1β to mature IL-1β (Fig. 4E). This demonstrates that FcγR cross-linking provides a robust priming ‘Signal 1’ for NLRP3 inflammasome assembly. To test the extent to which IL-1β production was dependent on NLRP3 in human macrophages, we performed NLRP3 protein knock-down using the ‘Trim-away’ technique (Fig. 4F) (Clift et al., 2017). This substantially abrogated IgG-induced macrophage IL-1β production, as did the addition of an NLRP3 inhibitor MCC950 (Fig. 4F). IL-1β production by human MDMs following combined stimulation of IgG IC and commensals or LPS was also reduced by the addition of MitoTEMPO, a mitochondrial ROS inhibitor (Fig. 4G, S4G). Together these data suggest that in UC, IgG-commensal ICs cross-link intestinal macrophage FcγRIIA, resulting in NLRP3-dependent IL-1β production.

To link these observations to the genetic variants associated with UC, we investigated the impact of the FcγRIIA-H/R131 SNP on macrophage IL-1β production. Following stimulation with IgG IC, we observed higher *IL1B* expression in MDMs obtained from subjects with the FcγRIIA-H/H131 genotype compared with FcγRIIA-R/R131 macrophages (Fig. 4H), demonstrating that this SNP, and the functional effect it confers on the FcγR A:I ratio, determines the magnitude of IL-1β induction in this context. Modeling FcγRIIA function *in vivo* is challenging as mice do not express FcγRIIA, and mice deficient in the orthologous receptor *Fcgr3* have a complete loss of activating signaling from this receptor, which does not reflect the graded functional effect of the FcγRIIA-H/R131 SNP. Our analysis of intestinal macrophages demonstrated that FcγR expression is dominated by the single inhibitory FcγRIIB in both mice and humans, and one functionally-homologous low-affinity activating FcγR (FcγRIII in mice and FcγRIIA in humans) (Fig. 2G, I). Therefore, we used mice with intact activating FcγR signaling, but a variable FcγR A:I ratio due to absent, wild type, or high inhibitory receptor expression allowing interrogation of the effect of graded activating FcγR signaling strength on intestinal inflammation (Fig. 4I). *In vitro,* IgG IC stimulation of BMDM from mice with a low FcγR A:I ratio due to macrophage-specific over-expression of FcγRIIB (Brownlie et al., 2008) (macrophage-transgenic; M-TG) resulted in lower *Il1b* induction compared to BMDM from WT mice, in contrast to BMDM from mice with a high A:I ratio due to FcγRIIB deficiency, in which *Il1b* induction was significantly higher than WT (Fig. 4J), in a manner analogous to the FcγRIIA-H/R131 variant (Fig. 4H, I). Similar results were observed for *Cxcl1* and *Cxcl2* (Fig. S4H). We concluded, therefore, that these mice represented a useful model to study the effects of differing FcγR A:I ratio *in vivo*.

**MNP FcγR A:I ratio modulates intestinal inflammation**

Compared with co-housed WT controls, *Fcgr2b*-/- mice subjected to aDSS had a more severe disease course, with impaired weight recovery from day 7 onwards (Fig. 5A), a timepoint at which there is significant induction of anti-commensal IgG (Fig. 1E). Similarly, in an independent experiment, *Fcgr2b*-/- mice had more severe disease than co-housed, littermate WT controls, with *Fcgr2b+/-* demonstrating an intermediate phenotype (Fig. S5A). WT mice reconstituted *Fcgr2b-/-* bone marrow also showed increased susceptibly to more severe disease following exposure to aDSS compared to mice reconstituted with WT bone marrow (Fig. S5B). Following aDSS treatment, *Fcgr2b*-/- mice had increased colon weights, spleen and lymph node enlargement (Fig. S5C), as well as marked infiltration of CD45+ cells into the colonic mucosa and submucosa compared to co-housed WT controls (Fig. 5B). This infiltrate included neutrophils (Fig. 5C), indicative of on-going inflammation 3 weeks after exposure to aDSS. Inflamed *Fcgr2b-/-* colons had higher levels of *Il1b* transcripts (Fig. S5D) and increased numbers of pro-IL-1β-expressing cells (Fig. S5E). We observed significantly higher proportions of pro-IL-1β+ colonic CX3CR1+ MNPs in *Fcgr2b-/-* mice compared to WT counterparts, particularly in newly recruited Ly6Chi monocytes and Ly6C+ MHC-II+ macrophages (Fig. 5D, E, S5F), with minimal pro-IL-1β expression in colonic neutrophils (Fig. S5G). Furthermore, flow-sorted colonic MHC-II+ MNPs isolated from *Fcgrb-/-* mice with aDSS had increased *Cxcl1* and *Cxcl2* transcripts compared to those obtained from co-housed WT mice (Fig. 5F), consistent with our observations *in vitro*.

Next, we investigated intestinal pathology in FcγRIIB-M-TG mice, with a low A:I ratio, and confirmed increased FcγRIIB expression on colonic MNPs, with no difference in activating FcγRs (Fig. S5H), nor in FcγRIIB expression on colonic neutrophils, DCs, B cells, or epithelial cells in FcγRIIB-M-TG compared with non-transgenic (N-TG) controls (Fig. S5I). Following exposure to aDSS, in contrast to *Fcgr2b*-/- mice,FcγRIIB-M-TG mice had a less severe clinical disease course compared with N-TG controls, with lower weight loss (Fig. 5G), colonic and MLN weight (Fig. 5H), a trend towards increased colon length (Fig. S5J), but no difference in spleen size (Fig. 5H). Colonic neutrophil infiltration was also significantly reduced in M-TG mice, consistent with improved disease resolution (Fig. 5I). There was a trend towards reduced global *Il1b*, *Cxcl1, Cxcl2,* and *Ccl2* transcripts within the colons of these mice (Fig. S5K), while pro-IL-1β protein was significantly reduced in intestinal CX3CR1+ MNPs in FcγRIIB-M-TG mice (Fig. 5J, K, S5L).

Since FcγRIIB modulates B cell activation threshold, we profiled B cell class-switching and commensal-reactive IgG responses in our mouse models. We observed an increase in frequency and count of class-switched B cells in the MLN and spleen of *Fcgr2b*-/- mice, but no difference in total anti-commensal IgG titer following aDSS (Fig. S5M, O). We observed no significant difference in class-switched B cells in the MLN of FcγRIIB-M-TG animals at day 21 post-aDSS compared to N-TG mice and similar levels of anti-commensal IgG titers between these two strains (Fig. S5N, O). Together, these data demonstrate that the differences observed in macrophage IL-1β and disease severity in mice with differing FcγR A:I ratio were not related to differences in the titers of anti-commensal IgG.

**MNP FcγR A:I ratio modulates intestinal type 17 immunity**

GWAS have implicated the IL-23/IL-17A cytokine axis in both CD and UC (Catana et al., 2015). IL-17A is expressed in healthy mucosa and contributes to intestinal homeostasis, but in excess may promote inflammation (Leppkes et al., 2009). Th17 cells are key producers of IL-17A within the intestine, and inhibition of these cells can reduce intestinal inflammation (Withers et al., 2016). As well as T cell-independent effects, a key biological effect of IL-1β is to induce the differentiation and maintenance of Th17 cells (Chung et al., 2009; Shaw et al., 2012). Given our data demonstrating that IgG IC potently induce IL-1β production in intestinal macrophages in mouse and human, and that IgG IC-stimulated colonic macrophages exhibit a Th17-polarising phenotype (Fig. S6A), we sought to determine whether macrophage FcγR A:I ratio was sufficient to impact intestinal type 17 immunity. Analysis of UC biopsies identified a significant positive correlation between *IL17A* and *FCGR2A* (Fig. S6B). Furthermore, IgG IC stimulation of inflamed colonic LPMC resulted in the production of type 17-associated cytokines, including IL-17, GM-CSF and IL-22 (Fig. 6A). *In vivo*, the magnitude of intestinal type 17 responses was determined by the FcγR A:I ratio; in *Fcgr2b-/-* mice , we observed an increase in colonic *Il17a, Csf2*,and *Il22* transcripts post-aDSS (Fig. 6B), while there was little change in global *Ifng* levels (Fig. S6C). Intracellular cytokine staining of colonic CD3ϵ+ T cell subsets (Fig. S6D) demonstrated a significant increase in the frequency (Fig. 6C) and absolute cell count (Fig. 6D) of both IL-17A+ γδ and αβ CD4+ T cell subsets in the recovery phase of aDSS colitis. We also observed a significant increase in IL-22 production by these T cell subsets (Fig. S6E, F), although the magnitude of this response was less than for IL-17A. Globally, there was a significant correlation between *Il1b* and type 17 cytokine genetranscript levels in inflamed colons (Fig. S6G), suggesting that FcγR-induced type 17 responses may be IL-1β-dependent.

In support of this, augmented IL-17A production by T cells in *Fcgr2b-/-* mice was completely abrogated by treatment with an anti-IL-1R1 blocking antibody (Fig. 6E, S6H), as was the increase in IL-22 (Fig. S6I). Conversely, in FcγRIIB-M-TG mice, there was a specific reduction in mucosal IL-17A-producing CD4+ αβ T cells and γδ T cells post-aDSS compared with N-TG controls (Fig. 6F, G). Little change in colonic B cells was observed following IL-1β blockade in *Fcgr2b-/-* mice (Fig. S6J), however, there was a reduction in the severity of colitis (Fig. 6H) and colonic neutrophil infiltration (Fig. 6I), directly implicating this pathway in detrimental immune responses driven by dysregulated FcγR signaling.

**Discussion**

IgG positive cells were first described in colonic biopsies from IBD patients more than 40 years ago by Brandtzaeg and colleagues (Baklien and Brandtzaeg, 1975). Despite subsequent confirmation of their commensal specificity (Macpherson et al., 1996), there has been little widespread acceptance that commensal-IgG immune complexes play a pathogenic role in UC. The subsequent identification of *FCGR2A\*A519G* (rs1801274) as the most-significant non-HLA genetic variant associated with UC in a Japanese GWAS (Asano et al., 2009) further supports this concept. This non-synonymous SNP results in an amino acid substitution that alters IgG binding affinity, the low affinity variant (FcγRIIA-R131) is protective in both candidate gene studies (odds ratio 0.70-0.84) (Weersma et al., 2010; Yang et al., 2011) and a meta-analysis of IBD GWAS (Jostins et al., 2012). Our study sheds light on the mechanisms underpinning these observations, demonstrating an increase in anti-commensal IgG and in the FcγR A:I ratio of mucosal immune cells in UC. The net functional effect of this is to lower the cellular activation threshold, rendering intestinal macrophages more readily activated by local IgG IC - an effect significantly offset by the low affinity FcγRIIA-R131.

The generic effects of cross-linking FcγR on monocyte/macrophage cytokine production have been widely studied over many years by ourselves and others (Clatworthy and Smith, 2004; Clynes et al., 1999), including in intestinal macrophages (Uo et al., 2013), demonstrating increases in TNF, IL-6, IL-1β, IL-10 and TL1A, to name but a few. Of these many potentially pathogenic cytokines, our study specifically identifies IL-1β as a key driver of IgG-associated inflammation in UC, and reveals the molecular mechanisms underpinning this.

IL-1β correlates with disease severity in patients with IBD (Ligumsky et al., 1990; Reinecker et al., 1993), but its role in intestinal immunity is complex. Mice deficient in IL-1β exhibit a severe non-healing disease post-DSS colitis, suggesting involvement in intestinal repair (Bersudsky et al., 2014). In contrast, IL-1β can promote chronic intestinal inflammation by inducing IL-17A-secreting innate lymphoid cells and Th17 cells (Coccia et al., 2012). Our data identify IL-1β as a key mediator of IgG/FcγR-associated intestinal inflammation and show that it may act to induce type 17 immunity via effects on Th17 cells, γδ T cells and, potentially, group 3 innate lymphoid cells (although we did not specifically examine the latter here). We also find that IL-1β may have Th17 cell-independent effects, consistent with previous reports, for example by promoting neutrophil and monocyte recruitment (Dinarello, 2009). Notably, we observed IL-1β-dependent IL-17A and IL-22 production in both γδ and CD4+ αβ T cells, implicating both innate and adaptive T cell subsets in FcγR-driven mucosal inflammation. It will be of interest in future studies to determine the contribution of IgG-induced FcγR signaling to type 17 immunity in other models of intestinal inflammation, particularly those with a dominant role for adaptive T cells, for example, following T cell transfer into *Rag*-/- mice. A careful description of Th17 cytokines in this modelwill be of clinical relevance, given reports that isolated IL-17A blockade can exacerbate intestinal inflammation (Hueber et al., 2012).

Although anti-commensal IgG, particularly anti-flagellin antibodies are present in CD, the association of FcγR variants with disease susceptibility is less robust than in UC (Jostins et al., 2012). Differential effects of genetic susceptibility loci in CD and UC are well recognized. For example, genetic variants that promote intracellular pathogen recognition and clearance, are associated with CD and not with UC (Abraham and Cho, 2006). In this context, it is notable that the FcγRIIA-R131 polymorphism increases susceptibility to a number of infections (Clatworthy, 2014), and that IgG and FcγRs promote defense against mycobacterial infection (Lu et al., 2016). Indeed, in mice, IgG and activating FcγRs are protective against the systemic spread of *Citrobacter rodentium*, an enteropathic bacteria (Masuda et al., 2008). Therefore, the deleterious effects of FcγRIIA-R131 on bacterial clearance might outweigh its beneficial, anti-inflammatory effects in CD.

Our data also have therapeutic implications, identifying IgG and activating FcγR signaling as potential therapeutic targets in UC. The only randomized controlled trial to investigate the use of B cell depletion in (treatment-resistant) UC has shown no benefit, but is substantially under-powered, studying *n* = 24 subjects (Leiper et al., 2011). Given randomized trials of drugs licensed for IBD treatment typically require the inclusion of hundreds of patients to demonstrate efficacy (Feagan et al., 2013; Sandborn et al., 2012), the question of whether B cell manipulation may be of benefit in UC remains to be addressed. Agents to target activating FcγR signaling may be of utility and include Syk (Spleen tyrosine kinase) inhibitors, which are currently being assessed in rheumatoid arthritis (Weinblatt et al., 2010) and glomerulonephritis (Ma et al., 2017). Alternatively, application of an FcγRIIB agonist (Bosques and Manning, 2016) in UC would potentially decrease the FcγR A:I ratio, an effect functionally analogous to the presence of the low affinity FcγRIIA-R131. Our data would suggest that targeting FcγR signaling in UC would have a potential advantage over single cytokine blockade due to its impact on the production of multiple pro-inflammatory cytokines and chemokines. Finally, it is notable that a trial of IL-1R1 blockade using anakinra in acute severe UC (ISRCTN43717130) is underway in the UK (personal communication, Raine and Kaser).

In summary, our study sheds light on the observed genetic association of FcγRIIA polymorphisms with UC, revealing the specific molecular mechanisms by which anti-commensal IgG augment inflammation, and identifying novel therapeutic targets.

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**Author contributions**

T.C.D. designed and performed experiments, analysed data, and co-wrote the manuscript. T.W.D. and J.R.F performed experiments and analysed data. R.J.M, D.C, A.F., B.S., C.J., L.I.L, S.C., and E.M. performed experiments. K.S., K.S-B. and M.P. provided human tissue samples. M.R.C. conceived the study, designed experiments, analysed data and co-wrote the manuscript.

**Declaration of interests**

The authors declare no competing interests.

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**Figure legends**

**Figure 1. Anti-commensal IgG is associated with the magnitude of intestinal inflammation.** (A) Analysis of human Ig heavy chain gene transcripts in healthy and UC colonic biopsies (data derived from Gene Expression Omnibus (GEO) dataset GSE9452). (B) IgG- and IgA1/2-bound SYBR greenhi bacteria in UC and household healthy control (HHC) stool samples; analyzed as household pairs (*n* = 6 per group). (C) Correlation of pooled IgG- and IgA1/2-bound bacterial levels with clinical activity index (CAI). (*n* = 12). (D) Murine colon luminal IgG and IgA levels following two cycles of DSS administration (cDSS), normalized to total protein content (*n* = 10 per group). Medians are indicated. (E) Quantification of IgG-bound bacteria in stool following a single acute course of 6-day 2% DSS administration (aDSS) or H2O, with (red) and without (black) paired serum pre-incubation (*n* = 6-9 per group). Medians are indicated. (F) Correlation of IgG-bound commensals (no serum) from pooled control and colitic mice at d21 post-aDSS with markers of colonic inflammation – length and neutrophil count (*n* = 20). In (D-F), data are pooled from two independent experiments. (G) Opsonization of commensal bacterial species with d21 aDSS serum or healthy control serum (d0) (*n* = 5 per group). Medians are indicated. Data are representative of two independent experiments. (H) Confocal image of control or inflamed colons from cDSS-treated mice (red = IgG; green = Ki67; white = phalloidin). Data representative of three independent experiments. (I, J) Quantification of IgG and IgA-expressing IgM- B cell subsets in murine colons (I) and colon-draining MLN (J) at d21 post-aDSS administration versus controls (d0) (*n* = 5 per group). For absolute cell count quantification plots, medians are indicated. Data are representative of three independent experiments. *P* values were calculated using limma with multiple correction using BH (A), ratio paired *t* test (B), linear regression analysis (C, F), or the nonparametric Mann-Whitney *U* test (D, E, G, I, J). \* *P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001; \*\*\*\* *P* < 0.0001. See also Figure S1 and Table S1.

**Figure 2. Activating FcγR signaling in intestinal inflammation.** (A) Analysis of the top significantly differentially expressed genes (log2 FC > 2) in human active UC mucosal biopsies compared to controls (data from GSE38713). (B) *FCGR* gene enrichment in inflamed active UC (*n* = 15) compared to healthy control (*n* = 13), remission (*n* = 8), and non-inflamed mucosal biopsies (*n* = 7). Data from GSE38713. (C) *FCGR* gene expression in colonic biopsies from UC patients refractory (*n* = 16) or responsive (*n* = 8) to infliximab treatment and healthy controls prior to treatment initiation (*n* = 6) (Data from GSE16879). Min. to max. box-and-whisker plots shown in (B) and (C). (D) Area under the receiver operator curve (AUUROC) analysis for *FCGR2A* expression in infliximab-refractory mucosal biopsies compared to infliximab-responsive biopsies. Data were derived as in C. (E) GSEA of the KEGG *FcγR-mediated phagocytosis* pathway in UC and healthy colonic biopsies (left) and cDSS-inflamed and healthy murine colons (bottom) (data from GSE38713, UC = 15, HC = 13) and GSE42768 (cDSS = 5, H2O = 5). (F) FcγR expression by murine colonic leukocytes at day 7 post-aDSS. Data are representative of three independent experiments. (G) qPCR of FcγR mRNA expression in flow-sorted murine colonic CX3CR1+ Ly6Clo MHC-IIhimacrophages, as identified in F. Data are representative of two independent experiments. (H) Single cell RNAseq of immune cell subsets in healthy human colon (data from GSE81861). The heatmap illustrates top 50 cell type-specific markers ranked by AUROC score. (I) qPCR of FcγR mRNA expression in flow-sorted human ileal CD14+CX3CR1+ SSCint macrophages. (J) Colonic “monocyte waterfall” subset quantification by flow cytometry at d7 post-aDSS versus controls (*n* = 3 per group). Flow plots of CD11b+ CX3CR1+ waterfall subsets (left) and quantification of absolute cell counts for the subsets shown (right). For absolute cell counts, medians are indicated. Data are representative of three independent experiments. (K) FcγR quantification of CX3CR1+ Ly6Clo MHC-IIhi macrophages, as shown in J, by flow cytometry. Means ± s.e.m. are indicated and normalized to the A:I ratio of control-treated mice. Data are representative of two independent experiments. *P* values were calculated using limma with multiple correction using BH (A-C), AUROC analysis (D, H), or Student’s two-tailed *t* test (J, K). \* *P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001; \*\*\*\* *P* < 0.0001. See also Figure S2 and Table S2.

**Figure 3. Mucosal FcγR expression correlates with IL-1β and CXCL8.** (A) Log2 FC comparison of differentially expressed cytokine and chemokine genes across two independent UC cohorts. Data from GSE38713 (UC = 15, HC = 13) and GSE59071 (UC = 74, HC = 11). (B, C) Correlation of *FCGR2A* expression with UC-associated cytokine/chemokine gene transcripts in n=85 mucosal biopsies (data from GSE59071). (D) Hierarchical clustering of chemokine/cytokine and *FCGR2A* gene expression in healthy controls, non-inflamed and inflamed UC patients (data derived from GSE38713). (E, F) Correlation of *Fcgr3* expression with *Il1b* (E) and candidate gene (F) expression in whole inflamed colonic tissue by qPCR (*n* = 16). Data are representative of two independent experiments. (G) *IL1B* and *CXCL8* expression in healthy human LPMC stimulated with Ova or Ova-IC for 16 h (*n* = 5 per condition). Means ± s.e.m. are indicated. Data are representative of two independent experiments. (H) IL-1β, CXCL1 and CXCL2 protein in supernatants of inflamed murine LPMCs stimulated with Ova or Ova-IC for 16 h (*n* = 3-9). Paired samples represent LPMCs from a single mouse. Data are pooled from two independent experiments. (I) Flow cytometry profiling of pro-IL-1β-expressing cells at d14 post-aDSS (*n* = 6). Medians are indicated. Data are representative of three independent experiments. (J) Pro-IL-1β expression by colonic CX3CR1+ MNP subsets and neutrophils in *Rag2-/-* mice treated with control or anti-flagellin-enriched serum IgG and 7-day aDSS or uninflamed H2O-treated controls (*n* = 5 per group). Medians are indicated. (K) Day 7 weight loss for aDSS-treated mice shown in J (*n* = 5 per group). Medians are indicated. *P* values were calculated using limma with multiple correction using BH (A), linear regression analysis (B, C, E, F), Student’s two-tailed *t* test (G), ratio paired *t* test (H, I), or the Kruskal-Wallis test with Dunn’s multiple comparisons test (J). \* *P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001; \*\*\*\* *P* < 0.0001. See also Figure S3.

**Figure 4. IgG-induced IL-1β production by human macrophages.** (A) Venn diagram of significant co-expressed genes between UC and Ova-IC-stimulated colonic macrophages (left) and IC-induced UC-associated cytokines and chemokines (right), as determined by sdef (human UC data from GSE38713, UC = 15, HC = 13). For macrophage stimulation, *n* = 4 per condition. (B) GSEA of inflammasome genes (left) and heatmap of selected core enrichment genes (right) in flow-sorted murine intestinal CX3CR1+ Ly6Clo MHC-IIhi macrophages stimulated with Ova/Ova-IC for 4h (*n* = 4 per condition). (C) IL-1β production by WT and *Nlrp3-/-* BMDMs primed with Ova/Ova-IC for 4h followed by 30 min ATP stimulation (\*\*\*\* WT versus *Nlrp3*-/- Ova-IC; #### WT Ova versus Ova-IC) (*n* = 3 per condition). Means ± s.e.m. are indicated. Data are representative of three independent experiments. ND = not detected. (D) IL-1β ELISA of human MDMs stimulated with plate-coated IgG (c-IgG) and intestinal commensals (Comm.) for 24h +/- anti-FcγRIIA IgG blocking antibody (*n* = 3 per condition). Means ± s.e.m. are indicated. Data are representative of two independent experiments. (E) qPCR of *IL1B* mRNA (left, *n* = 3 per condition) and Western blot of IL-1β in cell lysates and cell-free supernatants (right) from human MDMs as stimulated in D. For qPCR (left), means ± s.e.m. are indicated. Data are representative of two independent experiments. (F) The effect of NLRP3 inhibition on IgG-induced MDM IL-1β production. Western blot (left) and IL-1β production (left ELISA) by human MDMs stimulated as in D following NLRP3 “Trim-Away” or +/-NLRP3 inhibitor MCC950 (right ELISA) (*n =* 3 per condition). Means ± s.e.m. are indicated. (G) IL-1β production by human MDMs stimulated as in D +/- mitochondrial ROS inhibitor mitoTEMPO (*n* = 3 per condition). Means ± s.e.m. are indicated. Data are representative of two independent experiments. (H) Fold change in *IL1B* expression in Ova-IC-stimulated MDMs, normalized to R/R fold change (*n* = 6 per group). Data pooled from three independent experiments. Medians are indicated. (I) Schematic of FcγR A:I ratios with *FCGR2A* SNP (rs1801274) and murine transgenic models. (J) *Il1b* induction in WT, *Fcgr2b*-/-, and M-TG BMDMs stimulated with Ova/Ova-IC for 3h (*n* = 3 per condition). Means ± s.e.m. are indicated. Data are representative of three independent experiments. *P* values were calculated using the standard DESeq 2 method with multiple correction using BH (A, B), two-way ANOVA with Tukey’s multiple comparisons test (C, F, J), one-way ANOVA with Tukey’s multiple comparisons test (D, E, G), or parametric Student’s *t* test (H). \* *P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001; \*\*\*\* *P* < 0.0001. See also Figure S4.

**Figure 5. MNP FcγR A:I ratio modulates intestinal inflammation.** (A) Weight loss in co-housed sex-matched WT (black circles, *n =* 8) and *Fcgr2b*-/- (red circles, *n* = 9) mice following a single 6-day course of 2% DSS. Mean ± s.e.m. are indicated. Data representative of three independent experiments. (B) Confocal microscopy of colonic CD45+ leukocytes in mice treated as in A. Data representative of two independent experiments. (C) Flow cytometric quantification of colonic neutrophil frequency (top) and absolute neutrophil counts (bottom) in WT and *Fcgr2b-/-* colonsat d25 post-aDSS (*n* = 5-7 per group) or in healthy controls (day 0) (*n* = 3-5 per group). Medians are indicated. Data representative of three independent experiments. (D) Pro-IL-1β expression by colonic CD11b+ CX3CR1+ MNPs at d25 post-aDSS (*n* = 5-7 per group). (E) Frequency of pro-IL-1β expression within the Ly6Chi MHC-II- monocyte and Ly6C+ MHC-IIint macrophage populations as shown in aDSS (*n* = 5-7 per group) versus controls (d0) (*n* = 3-5 per group). Medians are indicated. Data representative of three independent experiments. (F) Chemokine mRNA levels in flow-sorted Ly6Clo MHC-IIhi colonic macrophages at d21 post-aDSS (*n* = 4-5 per group). Data normalized to WT mRNA levels. Medians are indicated. (G, H) Weight loss (G) and clinical colonic and lymphoid organ features (H) of co-housed sex-matched M-TG (green circles, *n* = 5-6) and N-TG littermate controls (black circles, *n* = 6) post-aDSS or in healthy controls (day 0 in H). Mean ± s.e.m. (weight loss) and medians (colon, MLN, and spleen weight) are indicated. Data representative of three independent experiments. (I) Flow cytometric quantification of colonic neutrophil frequency (top) and absolute neutrophil count (bottom) in N-TG and M-TG mice at d21 post-aDSS (*n* = 6 per group) versus controls (d0) (*n* = 5-6 per group). Medians are indicated. (J) Pro-IL-1β expression by colonic CX3CR1+ MNP subsets in N-TG and M-TG mice treated as in I (*n =* 5-6 per group). (K) Frequency (top) and absolute cell count (bottom) of pro-IL-1β-expressing MNP subsets in N-TG and M-TG mice treated as in I. Medians are indicated. Data representative of two independent experiments. *P* values calculated using a two-way ANOVA with Bonferroni’s multiple comparisons test (A, G), or the nonparametric Mann-Whitney *U* test (C-F, H-K). \* *P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001; \*\*\*\* *P* < 0.0001. See also Figure S5.

**Figure 6. MNP FcγR A:I ratio modulates intestinal type 17 immunity.** (A) Type 17-associated cytokine production by day 14-LPMCs stimulated with Ova/Ova-IC for 16 h (*n* = 5-9). Paired samples represent cells isolated from the same mouse. Data pooled from two independent experiments. (B) qPCR of type 17 cytokines in WT and *Fcgr2b*-/- whole colonic tissue after cDSS versus controls (*n* = 4-9 per group). Data normalized to uninflamed healthy colon. Medians are indicated. Data representative of two independent experiments. (C) Flow cytometry plots of colonic IL-17A-expressing T cell subsets in co-housed sex-matched WT and *Fcgr2b*-/- mice at d21 post-aDSS (*n* = 6-8 per group) versus controls (d0) (*n* = 3-5 per group). (D) Quantification of absolute numbers of colonic IL-17A-producing T cells shown in C. Medians are indicated. Data representative of three independent experiments. (E) Quantification of absolute numbers of colonic IL-17A-producing T cell subsets in co-housed WT and *Fcgr2b*-/- miceat d15 post-aDSS and weekly treatment with anti-IL-1R1 IgG blocking antibody or control IgG (*n* = 5-7 per group). Medians are indicated. Data representative of two independent experiments. (F) Colonic IL-17A-expressing T cell subsets in M-TG and N-TG littermate controls at d21 post-aDSS (*n* = 5-6 per group) versus controls (*n* = 5-6 per group). Data representative of two independent experiments. (G) Quantification of absolute cell counts of colonic IL-17A-producing T cell subsets as shown in F. Medians are indicated. (H) Weight loss of *Fcgr2b*-/- mice treated with control or anti-IL-1R1 IgG antibodies following aDSS treatment (*n* = 5-7 per group). Mean ± s.e.m. are indicated. Data representative of two independent experiments. (I) Colonic neutrophil infiltration in WT and *Fcgr2b*-/- mice treated as in H (*n* = 5-7 per group). Medians are indicated. *P* values were calculated using a ratio paired *t* test (A), the nonparametric Mann-Whitney *U* test (B-G, I), or a two-way ANOVA (H). \* *P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001; \*\*\*\* *P* < 0.0001. See also Figure S6.

**STAR Methods**

**CONTACT FOR REAGENT AND RESOURCE SHARING**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Menna R. Clatworthy (mrc38@cam.ac.uk).

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**Mouse strains**

All mouse lines used here are on a C57BL/6 background. *Fcgr2b-/-* mice were kindly provided by J. Ravetch (Rockefeller University) and S. Bolland (US National Institutes of Health, US National Institute of Allergy and Infectious Diseases (NIAID)) (Bolland and Ravetch, 2000). Macrophage transgenic (M-TG) and non-transgenic (N-TG) littermate controls were kindly provided by K.G.C. Smith. FcγRIIB overexpression was achieved using a construct in which FcγRIIB mRNA was placed under the control of the human CD68 promoter. NLRP3-deficient mice were kindly provided by Clare Bryant (University of Cambridge). C57BL/6, CD45.1+ C57BL/6 and *Rag2*-/- mice were obtained from Jackson Laboratories (Margate, UK) and maintained inhouse for several generations. For the generation of bone marrow chimeras, recipient CD45.1+ C57BL/6 mice were lethally irradiated (2 x 5.5 G) followed by immediate tail intravenous (i.v.) injection of 2 x 106 bone marrow cells from CD45.2+ C57BL/6 or CD45.2+ *Fcgr2b*-/- mice. Recipient mice were checked for reconstitution after 8 weeks prior to commencement of colitis experiments. For all *in vivo* colitis experiments, 6 to 14-week old sex-matched mice were used and mice were co-housed throughout the duration of experiments. Both male and female mice were used. All M-TG and N-TG experiments were performed on littermates. In the case of non-littermate controls, mice were co-housed for at least 3 weeks prior to the initiation of experiments. Mice were maintained in specific pathogen-free conditions at a Home Office-approved facility in the UK. All procedures were carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986.

**Human samples**

Human ileal tissue was obtained from deceased donors with prior ethical approval (REC: 15/EE/0152). Samples were obtained from 2 male and 3 female donors aged 19-77. UC (*n* = 6) and healthy control (*n* = 6) stool samples were obtained locally (Addenbrooke’s Hospital, Cambridge) with prior ethical approval (REC: 05/Q0108/355) from 6 male and 6 female donors and patient disease severity was scored using the Walmsley clinical activity index (CAI) (Table S1). Peripheral blood mononuclear cells (PBMCs) were collected from blood leukocyte cones or healthy volunteers (*FCGR2A* SNP study) with prior ethical approval from the local ethics committee (REC: 08/H0308/176). Blood was obtained from 6 male and 6 female donors aged 24 to 55.

**Commensal strains**

Commensal species were isolated from faeces of C57BL/6 mice and grown in LB medium at 37°C in a shaking incubator. *Parabacteroides distasonis* was grown in static culture in LB medium in an anaerobic cabinet.

**METHOD DETAILS**

**DSS-induced experimental colitis**

Colitis was induced the addition of 2 % (*w*/*v*) 36,000-50,000MW DSS (MP Biomedicals) to drinking water for 6 days (acute DSS (aDSS)). Mice were culled at various timepoints up to day 28 following aDSS, as described in the text/figure legends. In some experiments, mice were subjected to a second 6-day course of 2 % DSS after a two-week interval, termed chronic DSS (cDSS). Antibody was administered *in vivo* via i.p. injection (final volume, 200 μl sterile PBS). 1 mg InVivoMab anti-IL-1R1 IgG (JAMA-147; BioXCell) was given on day 0 and day 7 of a aDSS protocol. 0.5 mg anti-flagellin or control serum IgG was injected on day 0 of aDSS protocol. 0.5 mg rabbit anti-*Escherichia coli/Enterobactericeae* IgG (Abcam, ab137967) or PBS was injected on day 0 and day 3 of aDSS protocol.Colitis severity was monitored daily through changes in body weight, stool consistency, and intestinal hemorrhage. Moderate severity limits were imposed, with 20 % weight loss or two moribund characteristics judged to be the severity threshold. At experimental endpoints, colon, spleen, MLN and blood were harvested and colitis severity further assessed through morphological changes in organs. The spleen, MLN, and colon were weighed, and colon length measured from cecum to rectum to determine length. The tissues were then processed for histology, RNA extraction, or flow cytometric analysis.

**Flagellin immunization**

Flagellin immunization was carried out as previously described (Kobayashi et al., 2009). Briefly, C57BL/6 mice were hyperimmunized by two intraperitoneal (i.p.) injections with 10μg of flagellin from *Salmonella typhimurium* (InvivoGen) in Incomplete Freund’s Adjuvant (IFA) (Sigma Aldrich) two weeks apart. Mice were culled at four weeks and total serum IgG, as well as control serum IgG from unimmunized mice, was purified using Pierce Protein G purification columns (Thermo Fisher Scientific), as per the manufacturer’s instructions.

**Murine primary cell isolation**

Spleen, MLN, and colon were harvested and processed for single cell suspensions. Colons were dissociated from fat and luminal contents were gently removed. Tissues were opened longitudinally, cut into 0.5 cm pieces and washed by vortexing in ice-cold PBS with 10 mM HEPES. Tissue pieces were subsequently incubated with a stripping solution (RPMI-1640 medium containing 2 % (*v*/*v*) FCS, 10 mM HEPES, 1 mM DTT, and 5 mM EDTA) at 37˚C for two intervals of 20 min to remove epithelial cells, prior to enzymatic digestion in RPMI-1640 medium containing 1 mg/ml collagenase A (Sigma Aldrich) and 60 μg/ml DNase I (Roche). Tissue suspensions were mechanically dissociated and passed through a 70 μm cell strainer. Intestinal single cell suspensions were then harvested at the interface of a 40/80 % (*v*/*v*) Percoll (Sigma-Aldrich) gradient and washed thoroughly in ice-cold PBS containing 3 % (*v*/*v*) FCS before proceeding to further analysis. MLN and spleen suspensions were harvested by enzymatic digestion and mechanical tissue dissociation through a 70 μm filter. Splenic suspensions were subjected to red blood cell lysis (distilled H2O containing 0.83 % (*w*/*v*) NH4Cl, 0.1 % (*w*/*v*) NaHCO3, 100 μM EDTA) prior to washing twice in ice-cold PBS for analysis.

**Human primary cell isolation**

Human LPMC isolation was carried out in a similar way to murine LPMC isolation. Tissue samples were opened longitudinally and cleaned of luminal contents. The mucosa was manually dissociated from the muscular layers, cut into small pieces and washed by vortexing in ice-cold PBS containing 10 mM HEPES. Epithelial cells and intraepithelial lymphocytes were removed by incubation in stripping buffer, as above, at 37˚C for 1 h. The underlying tissues were then enzymatically digested for 1 h at 37˚C in RPMI-1640 medium containing 0.42 mg/ml Liberase (Roche) or 1 mg/ml Collagenase A (Sigma-Aldrich), and 60 ug/ml DNase I (Roche), vortexed, and mechanically dissociated using a Gentle-MACS machine (Miltenyi Biotech). Dissociated tissue was then passed through a 70 μm cell strainer and centrifuged for 10 min at 1300 rpm. Intestinal single cell suspensions were harvested at the interface of a 40/80 % (*v*/*v*) Percoll gradient and washed twice in ice-cold PBS containing 3 % (*v*/*v*) FCS before proceeding to downstream applications.

**Flow cytometry**

Single cell suspensions were blocked with 0.5 % (*v*/*v*) heat-inactivated mouse serum followed by extracellular staining for 1 h at 4°C with a combination of the following antibodies. Murine antibodies: B220 (RA3-6B2, Thermo Fisher Scientific), CD3ϵ (145-2C11, Thermo Fisher Scientific), CD4 (GK1.5, Thermo Fisher Scientific), CD11b (M1/70, Thermo Fisher Scientific), CD11c (N418, Thermo Fisher Scientific), CD138 (DL-101, Thermo Fisher Scientific), CD19 (6D5, Biolegend), CD38 (90, Thermo Fisher Scientific), CD45.2 (104, Thermo Fisher Scientific), CX3CR1 (SA011F11, Biolegend), EpCAM (G8.8, Thermo Fisher Scientific), F4/80 (BM8, Thermo Fisher Scientific), FcγRI (X54-5/7, Biolegend), FcγRIIB (AT130-2, Thermo Fisher Scientific), FcγRIII (275003, R&D systems), FcγRIV (9E9, Biolegend), IgA (SouthernBiotech), IgD (11-26, Thermo Fisher Scientific), IgG (SouthernBiotech), IgG1 (A85-1, BD Biosciences), IgG2b (R12-3, BD Biosciences), IgG3 (R40-82, BD Biosciences), IgM (II/41, Thermo Fisher Scientific), Ly6C (HK1.4, Thermo Fisher Scientific), Ly6C/G (RB6-8C5, Thermo Fisher Scientific), MHC-II (M5/114.15.2, Thermo Fisher Scientific), TCR beta (H57-597, Thermo Fisher Scientific), and TCR gamma/delta (GL3, Biolegend). Human antibodies: CD14 (61D3, Thermo Fisher Scientific), CX3CR1 (2A9-1, Thermo Fisher Scientific), FcγRI (10.1, Thermo Fisher Scientific), FcγRIIA/B (CD32, Thermo Fisher Scientific), FcγRIIIA/B (eBioCB16, Thermo Fisher Scientific), IgA1/2 (G20-359, BD biosciences), and IgG (HP6017, Biolegend). Antibodies were used at a dilution of 1:200 in PBS. Viability staining was performed with LIVE/DEAD Fixable Aqua Dead Cell Stain kit (Thermo Fisher Scientific) for 20 min at room temperature. For biotinylated primary antibodies, secondary staining with streptavidin-conjugated PE (Thermo Fisher Scientific) or APC-eFluor780 (Thermo Fisher Scientific) was performed for 20 min at 4°C at a dilution of 1:300 in PBS. For intracellular cytokine staining, cells were incubated in RPMI-1640 medium containing 10 % FCS, 1X penicillin-streptomycin (both Sigma-Aldrich), and 1X Brefeldin A (Thermo Fisher Scientific) solution for 3 h at 37°C, prior to fixation and permeabilization using the Intracellular Fixation and Permeabilization Buffer Set (Thermo Fisher Scientific) as per the manufacturer’s instructions. Staining was carried out for 1 h at room temperature using a combination of the following antibodies: IL-17A (TC11-18H10.1, Biolegend), IL-22 (IL22JOP, Thermo Fisher Scientific), and pro-IL-1β (NJTEN3, Thermo Fisher Scientific). All antibodies were used at a 1:100 dilution. Cell counting was performed using 123count eBeads (Thermo Fisher Scientific). Flow cytometry data collection was performed on a Fortessa cytometer (BD biosciences) and data was analyzed using FlowJo software (Tree Star Inc.).

**Flow sorting**

Murine intestinal macrophages were flow-sorted as live CD11b+ CX3CR1+ Ly6Clo MHC-IIhi cells. Human ileal macrophages were flow-sorted as SSCint CX3CR1+ CD14+ cells. Cell sorting was performed on FACS Aria Fusion (BD biosciences), iCyt Synergy (Sony Biotechnology Inc.), and MoFlo (Beckman Coulter) cell sorters. Data were analyzed using FlowJo software (Tree Star Inc.).

**Microbial flow cytometry**

For murine stool samples, colonic fecal contents were homogenized in sterile PBS, briefly centrifuged at 1,000 rpm to remove large aggregates, and the resulting supernatant was washed twice in sterile PBS by centrifugation for 1 min at 8,000 rpm. At harvesting of commensal isolates, bacterial cultures were centrifuged for 10 min at 4000 rpm and used for downstream analyses. For all samples, bacterial pellets were resuspended in sterile PBS containing 1:50 dilution of mouse serum or PBS alone in 96-well v-bottom plates (Thermo Fisher Scientific) and incubated for 20 min at 4°C. For transgenic mouse serum anti-commensal IgG comparison, stool from *Rag2*-deficient mice was used, which lacks endogenous anti-commensal IgG. Human stool samples were processed as above, plated in 96-well v-bottom plates (Thermo Fisher Scientific) in sterile PBS, and centrifuged at 3,000 rpm for 10 min in a bench-top Sorvall centrifuge. For both human and murine samples, bacterial pellets or serum-opsonized bacteria were then resuspended in 50 μl sterile ice-cold PBS containing anti-mouse/human IgA-PE or anti-mouse/human IgG-Alexa 647 antibodies and stained for 20 min at 4°C. Cells were washed and resuspended in PBS or fixative containing 1:10,000 SYBR Green (Thermo Fisher Scientific) and analyzed by flow cytometry. Fecal and serum samples were paired from the same mouse, unless otherwise stated, and SYBR Green-high events were analyzed.

**Serum anti-commensal IgG ELISA**

For serum anti-commensal IgG analysis, colonic fecal contents were processed as described above. Bacteria were homogenized using BugBuster 10X protein extraction reagent (Novagen), centrifuged at 20,000 *g* for 10 min, and the supernatant recovered for a crude commensal bacterial antigen preparation. Protein concentration was determined using the Pierce BCA protein assay kit (Thermo Fisher Scientific). Subsequently, 96-well Nunc ELISA plates (Thermo Fisher Scientific) were coated with 5 μg/ml commensal antigen preparation overnight at 4°C, washed extensively, and murine sera incubated in doubling dilutions for 4 h at room temperature. For small volumes of sera, samples were incubated at a 1:150 dilution. In the case of serum anti-flagellin IgG detection, 96-well Nunc plates were coated overnight with 200 ng/ml flagellin purified from *Salmonella typhimurium* (InvivoGen). Commensal antigen-specific IgG was detected using a goat anti-mouse IgG-horseradish peroxidase (HRP) conjugated antibody (Thermo Fisher Scientific, 1:10000 dilution), and TMB peroxidase substrate (BD biosciences). After 15-20 min, the reaction was quenched with 1 M Na2SO4 and the optical densities measured at 450 nm using a CLARIOstar spectrophotometer (BMG Labtech).

**Luminal IgG ELISA**

Luminal contents were extruded using bicarbonate buffer (15 mM Na2CO3, 35 mM NaHCO3) containing cOmplete protease inhibitor (Roche). 96-well Nunc ELISA plates (Thermo Fisher Scientific) were coated with primary goat anti-murine IgG or goat anti-murine IgA antibodies (catalogue numbers 1037-01 and 1040-01, respectively; SouthernBiotech) for 16 h at 4°C. Plates were extensively washed and incubated with luminal suspensions in serial dilutions for 4 h at room temperature. Bound IgG and IgA were detected using secondary goat anti-murine IgG and goat anti-murine IgA antibodies conjugated to HRP (catalogue numbers 1037-05 and 1040-05, respectively; SouthernBiotech) and TMB peroxidase substrate (BD biosciences). Ig concentration was determined using a standard curve of murine IgG (I5381-5mg; Sigma-Aldrich) or murine IgA (14-4762-81; Thermo Fisher Scientific). Ig levels were normalized to total luminal protein content, as determined using the Pierce BCA protein assay kit (Thermo Fisher Scientific).

**Macrophage and intestinal immune cell culture**

For murine BMDMs, bone marrow was flushed from the femur and tibia of mice using ice-cold sterile PBS and the subsequent cell suspension treated with red cell lysis buffer. Treated cells were then washed in ice-cold sterile PBS. BMDMs were generated by incubation of bone marrow cells in RPMI-1640 medium containing 10 % FCS and 1X penicillin-streptomycin (both Sigma-Aldrich) (referred to as complete RPMI) supplemented with 100 ng/ml murine macrophage colony-stimulating factor (M-CSF; Peprotech). M-CSF-supplemented culture medium was replaced on day 3 and BMDMs were harvested on day 5-6. BMDMs were primed with 20 ng/ml murine granulocyte-macrophage colony-stimulating factor (GM-CSF; Peprotech) for 16 h prior to stimulation. For human MDM culture, diluted blood was overlaid on Histopaque 1077 (Sigma-Aldrich) in a 50 ml centrifuge tube and centrifuged for 20 min at 2,000 rpm at room temperature. The PBMC layer was removed and resuspended in cold complete RPMI. PBMCs were incubated in complete RPMI medium supplemented with 100 ng/ml human M-CSF (Peprotech). Additional M-CSF-supplemented media was added at day 3 and adherent macrophages were harvested at day 6.

Flow-sorted intestinal MNPs were plated in complete RPMI-1640 medium at a density of 0.5-1 x 105 cells per well in 96-well round-bottom plates (Sigma-Aldrich) prior to stimulation. LPMC suspensions were plated in complete RPMI-1640 medium at a density of 5x105-1x106 cells per well in 24-well plates (Sigma-Aldrich) prior to *ex vivo* stimulation with Ova-IC or Ova. Cells were then washed extensively, and analyzed by qPCR, ELISA, and flow cytometry.

**IgG stimulation**

For IgG IC stimulation, model Ova-IC was generated by opsonization of 40 μg/ml endotoxin-free ovalbumin (Ova; Hyglos) with 1.2-2 mg/ml polyclonal rabbit anti-Ova IgG antibody in serum (Sigma-Aldrich) at 37°C for 1h. Immune cells were stimulated in complete RPMI with Ova, anti-Ova serum, or Ova-IC at 37°C in a 5 % CO2 incubator for 4 h for RNA analysis, including RNAseq and quantitative PCR (qPCR), or 16 h for cytokine and chemokine ELISAs. For BMDM IL-1β production, 5 x 105 murine BMDMs were stimulated with Ova or Ova-IC for 4 h at 37°C followed by 30 min stimulation with 2.5 mM ATP (Sigma-Aldrich).

For human c-IgG stimulation, 96-well high-affinity Nunc MaxiSorp plates (Thermo Fisher Scientific) were coated with 2 μg/ml human IgG (Sigma-Aldrich) overnight at room temperature. Plates were blocked with PBS containing 10 % FCS for 1 h. Macrophages were plated at 0.5-1x105 cells per well in triplicate in complete RPMI and additionally stimulated with 100 ng/ml LPS from *E. coli* (Sigma-Aldrich) or fecal commensal extract from healthy human donors harvested as above and outlined previously (Seo et al., 2015). Supernatants were harvested after 24 h and analyzed by ELISA. In certain experiments, macrophages were pre-incubated for 30 min at 37°C with 20 μg/ml anti-FcγRIIA blocking antibody (R&D systems) and subsequently maintained at 5 μg/ml in culture with additional stimuli. Mitochondrial ROS were blocked using 0.5 mM MitoTEMPO (Sigma-Aldrich).

**Trim-Away**

Human MDMs were electroporated with PBS, mouse anti-GFP (9F9.F9; Abcam) or mouse anti-NLRP3 (Cryo-2; Adipogen) IgG antibodies. All antibodies used for electroporation were passed through Amicon Ultra-0.5 100 kDa centrifugal filter devices (Millipore) to remove traces of azide and replace buffer with PBS. All antibodies were diluted to 0.6 mg/ml in PBS prior to electroporation. Antibody electroporation was performed using the Neon Transfection System (Thermo Fisher Scientific). MDMs were washed with PBS and resuspended in Buffer R (Thermo Fisher Scientific) at a concentration of 1.4 x 108 cells/ml. For each electroporation reaction 1.4 x 106 cells (10 µl) were mixed with 2 µl of antibody or PBS. The mixture was taken up into a 10 µl Neon® Pipette Tip (Thermo Fisher Scientific) and electroporated using the following settings: 1400V, 20 ms, 2 pulses. Electroporated cells were transferred to growth medium without antibiotics.

**Immunoblotting**

MDMs were plated and stimulated as indicated previously in serum-free medium. 500 ml of supernatants was collected and precipitated using Methanol/Chloroform extraction. 500 ml MeOH and 125 ml Chloroform was added to 500 ml cell-free supernatant, vortexed briefly and spun at 13 000 g for 5 mins. The upper layer was removed and a further 500 ml MeOH added to each sample, vortexed and spun at 13 000 g for 5 mins. The supernatant was completely aspirated and the protein pellet was resuspended directly in NuPAGE LDS Sample buffer with 100 mM DTT and heated at 95°C for 10 mins. MDMs were lysed in RIPA buffer (CST-9806) supplemented with a protease inhibitor cocktail (Roche), spun at 14000 g for 10 min and cleared lysates mixed with NuPAGE LDS Sample Buffer and heated at 95°C for 10 mins. Samples were run on NuPAGE 4 %–12 % Bis-Tris gels (Thermo Fisher) and transferred onto nitrocellulose membrane. Antibody incubations were performed in PBS with 5 % (*v*/*v*) milk and 0.1 % (*v*/*v*) Tween-20. The primary antibodies used were goat anti-IL-1β IgG antibody (BAF201, R&D Systems, 1:500), mouse anti-NLRP3 (Cryo-2; Adipogen; 1:500), rabbit anti-ASC (AL177; Adipogen; 1:500), mouse anti-TRIM21 (D-12; Santa Cruz Biotechnology; 1:500) and rabbit anti-COXIV (LI-COR; 1:5000). HRP-coupled secondary anti-goat (Santa Cruz, sc-2056), anti-mouse (Dako), anti-rabbit (Thermo Fisher Scientific), and anti-β-actin (Sant Cruz, sc-47778) antibodies were detected by enhanced chemiluminescence (Amersham, GE Healthcare) and X-ray films.

***FCGR2A* genotyping**

DNA was extracted from whole blood of healthy volunteers using the Quick-DNA Universal kit (Zymo Research) as per the manufacturer’s instructions. Genotyping was performed using the TaqMan Genotyping Master Mix (Thermo Fisher Scientific) and SNP Genotyping Assay probes for *FCGR2A* SNP rs1801274 (Thermo Fisher Scientific) as per the manufacturer’s instructions by qPCR.

**RNA extraction and reverse transcription**

RNA extraction was carried out using commercially available kits as per the manufacturer’s instructions. QIAGEN RNeasy micro kits were used for cell numbers below 5 x 105. The PureLink RNA mini kit (Thermo Fisher Scientific) was used for cell numbers over 5 x 105. For whole tissue RNA extraction, tissue pieces were first disrupted using a Precellys 24 Homogenizer (Bertin Instruments), before extraction using the PureLink RNA mini kit (Thermo Fisher Scientific). RNA concentration and purity were determined using a NanoDrop spectrophotometer (Thermo Scientific) prior to cDNA synthesis using a High-Capacity RNA-to-cDNA kit (Applied Biosystems).

**Quantitative polymerase chain reaction**

All qPCR was carried out in triplicate with Taqman reagents and the following pre-designed TaqMan Gene Expression Assay primers and probes (Thermo Fisher Scientific). Murine primers: *Ccl2* (Mm00441242\_m1), *Csf2* (Mm01290062\_m1), *Cxcl1* (Mm04207460\_m1), *Cxcl2* (Mm00436450\_m1), *Fcer1g* (Mm02343757\_m1), *Fcgr1* (Mm00438874\_m1), *Fcgr2b* (Mm00438875\_m1), *Fcgr3* (Mm00438882\_m1), *Fcgr4* (Mm00519988\_m1), *Gapdh* (Mm99999915\_g1), *Hprt* (Mm03024075\_m1), *Ifng* (Mm01168134\_m1), *Il1b* (Mm00434228\_m1), *Il6* (Mm00446190\_m1), *Il10* (Mm01288386\_m1), *Il17a* (Mm00439618\_m1), *Il22* (Mm01226722\_g1), *Il23a* (Mm00518984\_m1), *Tnf* (Mm00443258\_m1), and *Tnfsf15* (Mm00770031\_m1). Human primers: *CXCL8* (Hs00174103\_m1), *FCER1G* (Hs00175408\_m1), *FCGR1B* (Hs02341825\_m1), *FCGR2A* (Hs01013401\_g1), *FCGR2B* (Hs01634996\_s1), *FCGR3A* (Hs02388314\_m1), *FCGR3B* (Hs04334165\_m1), *GAPDH* (Hs02786624\_g1), *HPRT1* (Hs02800695\_m1), and *IL1B* (Hs01555410\_m1). qPCR was carried performed on the Viia 7 PCR machine (Life Technologies). Gene expression was normalized to *Gapdh* or *Hprt* using the 2-ΔCt. The 2-ΔΔCt method was used for normalization between experimental conditions and genotypes.

**Cytokine/chemokine ELISA**

Quantification of human and murine cytokines and chemokines in culture supernatants was carried out using commercially available R&D systems Duoset ELISA kits, as per the manufacturer’s instructions.

**Immunofluorescence**

Intestinal tissues were fixed with 1 % (*w*/*v*) paraformaldehyde (Electron Microscopy Services) in PBS for 16 h, washed with PBS, and equilibrated in 30 % (*w*/*v*)sucrose for a further 16 h. Tissues were then frozen at -80°C in Optimal Cutting Temperature (OCT) embedding medium (Thermo Fisher Scientific). Cryostat sections were cut at a thickness of 20-30 μm, air dried for 1 h, then rehydrated for 10 min in PBS and blocked with a 0.1 M Tris solution containing 1 % (*w*/*v*) mouse serum, 1 % (*w*/*v*) bovine serum albumin (BSA), and 0.1 % (*w*/*v*) Triton X-100 for 1 h at room temperature. Sections were stained overnight at 4°C with a combination of the following antibodies in blocking buffer at a 1:100 dilution: CD45.2 (104; Thermo Fisher Scientific), IgG (SouthernBiotech), and Ki67 (SolA15; Thermo Fisher Scientific). Additionally, actin was stained in certain experiments using Phalloidin dyes (Thermo Fisher Scientific) at a 1:200 dilution.Confocal imaging was carried out on a Leica SP8 confocal microscope. Images were analyzed using Imaris software (Bitplane).

**RNAseq sample preparation**

Flow-sorted CX3CR1+ CD11b+ Ly6Clo MHC-II+ macrophages were plated at a density of 1 x 105 cells per well in 96-well round-bottom plates and stimulated with Ova or Ova-IC for 4 h at 37°C in a 5 % CO2 incubator. Following stimulation, cells were transferred into 750 μl RLT plus buffer (QIAGEN). Samples were immediately vortexed, snap frozen on dry ice and stored at -80°C. RNA was extracted from cell lysates using the RNeasy plus micro kit (QIAGEN) as per the manufacturer’s instructions. Optimal DNA depletion columns (QIAGEN) were used to remove contaminating genomic DNA. Purified RNA was eluted in nuclease free water (Ambion) and stored at -80°C.Quality and concentration of the purified RNA was assessed using an RNA pico chip (Applied Biosystems) using a Bioanalyzer 2000 (Applied Biosystems) as per the manufacturer’s instructions. For all RNAseq experiments, samples had an RNA integrity number greater than 8, indicating minimal degradation of the RNA.For the preparation of libraries, SMARTer stranded total RNA-Seq mammalian pico input kit (Takara) was used as per the manufacturer’s instructions. To produce the libraries, 1.5-3.55 ng of total RNA was used and libraries were amplified for 14 cycles of PCR. Library size was assessed using 1 μl of undiluted final libraries with a High Sensitivity DNA chip (Applied Biosystems) using a Bioanalyzer 2000 (Applied Biosystems) as per the manufacturer’s instructions. Library concentration was quantified by PCR using 1/10000 dilution of the library in nuclease free water (Ambion) with ROX low KAPPA library quantification kit (KAPPA Biosystems). Libraries were pooled at an equimolar concentration with up to 12 libraries per pool.

**RNA sequencing and analysis**

Sequencing of the libraries was carried out using a Hiseq 2500 (Illumina) on a 2x100bp sequencing run with 1 pool per flow cell lane. Sequencing was carried out at Genewiz (NJ, USA).Pooled libraries were de-multiplexed by Genewiz using Casava (Illumina) before transfer of the data to the University of Cambridge. Fastq files were trimmed of the first 3 nucleotides of the R1 strand and contaminating adaptor sequences and poor-quality bases removed (bases with a phred 33 score of <30) using trimgalore! (Babraham bioinformatics) and quality of the resulting files was assessed using FastQC (Babraham bioinformatics). Fastq files were aligned to the mm10 genome (Downloaded from https://genome-euro.ucsc.edu/cgi-bin/hgGateway?db=mm10&redirect=manual&source=genome.ucsc.edu on 18th January 2016) using hisat2. All analysis was carried out using R version 3.2.4. Reads were counted and assigned to genes using the Featurecount function from the RSubread package. Differential expression analysis was carried out using DESeq2 using a linear model with an appropriate design matrix following the default workflow. Resulting figures were plotted using ggplot2 and heatmap.2 from the gplots package. GSEA was performed for RNAseq data by first assigning a rank metric to each gene using the following formula:

Rank metric = 1(*P* value + 1x10-300)\*(|LFC|/LFC)

GSEA was then run using GSEA 2.1 using the pre-ranked option with the classic setting against either gene sets from the molecular signature database or custom gene sets indicated in the text. FcγR gene lists were obtained from the Molecular Signature Database (MSigDB; http://software.broadinstitute.org/gsea/msigdb). Gene ontology analysis was carried out using TopGo. Selected populations were compared for enrichment to a gene universe which contained only genes which had a possibility of being present in both UC microarray and macrophage Ova-IC RNAseq groups. Enriched GO terms were identified using a Fisher elim method.

**Microarray**

Publicly available microarray datasets were downloaded from GEO (https://www.ncbi.nlm.nih.gov/geo/) along with appropriate chip annotation data. All analyses were carried out using R. All datasets were downloaded as raw intensity matrices. Data was normalized using RMA and limma. Probes were reduced to one probe per gene by selecting the probe with the greatest variance across the samples using the gene filter package. Differential expression was varied out using limma with an appropriate design matrix. GSE59071 (Vanhove et al., 2015), GSE38713 (Planell et al., 2013) and GSE9452 (Olsen et al., 2009) datasets were used for human UC analysis, GSE16879 (Arijs et al., 2009a; Arijs et al., 2009b) for infliximab-resistant UC analysis (see also Table S2), GSE42768 (Breynaert et al., 2013) for analysis of murine DSS-induced colitis, and GSE49109 for *C. rodentium* infection analysis (Marchiando et al., 2013). The *IGH* expression score was calculated as a sum of the normalized log2-transformed expression values of all *IGH*-containing probes in each sample. AUROC analysis was performed on normalized log2-transformed expression values of *FCGR2A* or the *IGH* expression score using GraphPad Prism 6. GSEA was performed as for RNAseq without pre-ranking against either gene sets from the molecular signature database or custom gene sets indicated in the text.

**Hierarchical clustering**

R and the base stats package were used for all calculations. Plots were generated using the package dendextend. For clustering analysis, the RMA normalized intensity for the genes of interest (all cytokines plus *FCGR2A*) were selected and a distance matrix calculated using Euclidean distance using the function “dist”. The samples were subsequently hierarchically clustered using the function “hclust” using the complete method. To define clusters, the tree was cut into k clusters at a given height based on visual interpretation of the dendrogram (typically k=4-5). The cluster containing *FCGR2A* was examined further.

**Single cell RNAseq**

Publicly available normal colonic mucosa single cell RNAseq raw count data (Li et al., 2017) was acquired from GEO (GSE81861). Data was analyzed in R using the Seurat package. Genes with very low expression were discarded (average counts < 0.4). Data was log-normalized using global scaling, and AUROC test was used to generate a ranked list of cell type-specific genes and associated scores. Peripheral blood mononuclear cell raw count data (Zheng et al., 2017) was acquired from the 10X genomics data portal (<https://support.10xgenomics.com/single-cell-gene-expression/datasets>) – Donor A 68K PBMC. Data was log-normalized using global scaling in the Seurat package. Thereafter we detected highly variable genes with average log expression values between 0.01 and8 using the FindVariableGenes function in Seurat. We extracted the top 20 cell loadings after principal components analysis on the expression matrix subsetted to variable genes and generated a UMAP embedding from these loadings. We clustered the resulting k-nearest neighbor graph using Louvain clustering with default settings and annotated clusters based on marker genes defined by the AUROC test. The top 50 marker genes for each annotated cell type were used to calculate a rank-based enrichment score for single cells in the gut dataset, using the AUCell package (Aibar et al., 2017).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Statistical analysis was performed using GraphPad Prism software or R. For *in vivo* colitis experiments, comparison between experimental groups was performed using a nonparametric Mann-Whitney-U test, unless otherwise stated, and medians are indicated. For *in vitro* stimulation experiments, mean ± standard error of mean (s.e.m.) are shown and a parametric Student’s two-tailed *t* test or two-way ANOVA with Tukey’s multiple comparisons test was used, unless paired samples were used, where a ratio paired *t* test was used. For correlations of RNA expression levels, linear regression analysis was used. For RNAseq bioinformatics analyses, *P* values were calculated using the standard DESeq 2 method with multiple correction using BH. For microarray experiments, *P* values were calculated using the limma package with multiple correction using BH. \* *P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001; \*\*\*\* *P* < 0.0001. Sample sizes (*n*) for all shown data can be found in the figure legends. *In vitro* stimulations were performed in triplicate, unless stated, and sample sizes for *in vivo* experiments were determined based on initial experiments.

**DATA AND SOFTWARE AVAILABILITY**

The accession number for the data in this paper is GEO: GSE109040.