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CSF-1 Dependence of Paneth Cell Development in the Mouse Small Intestine

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Abstract

Background & Aims—Paneth cells (PCs) secrete defensins and antimicrobial enzymes that contribute to innate immunity against pathogen infections within the mucosa of the small intestine. We examined the role of colony stimulating factor-1 (CSF-1) in PC development.

Methods—CSF-1-deficient and CSF-1 receptor (CSF-1R)-deficient mice and administration of neutralizing anti-CSF-1R antibody were used to study the requirement of CSF-1 for the development of epithelial cells of the small intestine. CSF-1 transgenic reporter mice and mice that express only the membrane-spanning, cell-surface CSF-1 isoform were used to investigate regulation by systemic versus local CSF-1.

Results—Mice deficient in CSF-1 or CSF-1R had greatly reduced numbers of mature PCs. PCs express the CSF-1R and administration of anti-CSF-1R antibody to neonatal mice significantly reduced the number of PC. Analysis of transgenic CSF-1 reporter mice showed that CSF-1-expressing cells are in close proximity to PCs. CSF-1/CSF-1R-deficient mice also had reduced numbers of the proliferating epithelial cell progenitors and lamina propria macrophages. Expression of the membrane-spanning, cell-surface CSF-1 isoform in CSF-1-deficient mice completely rescued the deficiencies of PC, proliferating progenitors and lamina propria macrophages.

Conclusions—These results indicate local regulation by CSF-1 of PC development, either directly, in a juxtacrine/paracrine manner, or indirectly, via lamina propria macrophages. Therefore, CSF-1R hyper-stimulation could be involved in hyperproliferative disorders of the small intestine, such as Crohn's disease and ulcerative colitis.

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INTRODUCTION

The small intestine (SI) progenitor cells, derived from stem cells in the crypt region, are responsible for populating the intestinal epithelium, giving rise to the three different epithelial cell types of the villi.^{1–5} These are the enterocytes, which secrete hydrolases, absorb nutrients and comprise more than 80% of villus epithelial cells,⁶ goblet cells that provide a protective mucous lining,^{5, 7} and rare enteroendocrine cells that secrete hormones, including serotonin, secretin and substance P.^{8, 9} The crypt stem cells also give rise to the Paneth cells (PC), which reside in the crypts and are the longest-lived cell type, surviving 18–22 days before degenerating and being phagocytosed by neighbouring cells. Each crypt contains 1–4 PCs, located below the stem cell zone (estimated to contain 4–6 stem cells^{2, 10}) in the crypt base.^{11–14} However, recent studies with the stem cell marker, Leucine-Rich G-coupled receptor gene (*Lgr-5*) indicate that SI stem cells intermingle with PC at the crypt bases.¹⁵

PCs are major components of innate immunity in the gut.^{16–18} They produce a spectrum of anti-microbial peptides and proteins^{16, 17} including the α -defensins (cryptdins),¹⁹ matrilysin or matrix metalloproteinase-7 (MMP-7),²⁰ synovial (type II) phospholipase A2²¹ and lysozyme.²² In response to bacteria, lipopolysaccharide and cholinergic stimulation associated with feeding, PCs discharge granules containing these mediators into the crypt lumen.^{17, 22} PCs react to bacterial invasion by releasing α -defensins in ample quantity to kill them.²³ However, the complexity of PC granule composition and the triggering of granule release by multiple stimuli, suggest that PCs may have additional functions, including regulation of intestinal inflammation, digestion, detoxification, stem cell protection and crypt development.¹⁷

CSF-1 regulation has been characterized in the CSF-1-deficient *osteopetrotic* (*Csf1^{op}*) mutant mouse,²⁴ which possesses an inactivating mutation in the CSF-1 gene^{25–28} and in mice with a targeted deletion of the sole CSF-1 cellular receptor, CSF-1R (*Csf1r^{-/-}/Csf1r^{-/-}*).^{29, 30} These studies showed that CSF-1 is the primary regulator of macrophage,²⁵ osteoclast³⁰ and Langerhans³¹ cell production and that all of the effects of CSF-1 are mediated via the CSF-1R.²⁹ Immunohistochemical staining for the macrophage marker, F4/80, demonstrated that interstitial macrophage numbers are reduced in the SIs of the *Csf1^{op}/Csf1^{op}* (*Csf1^{op/op}*) mice compared with their wild type (WT) littermates.²⁵ Because of the physical proximity of interstitial macrophages to the intestinal epithelium, we investigated the role of CSF-1 and the CSF-1R in the development of the SI. We found that the absence of either CSF-1 or CSF-1R causes abnormal SI organization, reduced expression of *Lgr5*¹⁵ and *Cyclin D1*, and a reduction in the number of differentiated epithelial cells. In particular, there is a dramatic reduction in PCs due to local regulation of their development and/or survival by the CSF-1.

MATERIALS AND METHODS

Mice

Csf1^{op/+24} and *Csf1r^{-/+29}* mice, backcrossed on the FVB/NJ background for at least 10 generations,³⁰ were used to generate homozygous mutant and WT (+/+) control mice. *Csf1^{op/op}*; *TgCS5/+* mice, exclusively expressing the cell-surface isoform of CSF-1 from the TgN(CSCSF1)5Ers (TgCS5) transgene³⁰ and *TgN(Csf1-Z)9Ers* homozygote (*TgZ9/TgZ9*) mice expressing β -gal,³² which in both cases have transgene expression driven by the same *Csf1* promoter and first intron regulatory region, were also on the FVB/NJ background. The generation, genotyping and husbandry of these mice have been described previously.^{29, 30, 32} For the anti-CSF-1R antibody treatment, each injection comprised 100 μ l of 10 mg/ml of either rat anti-mouse CSF-1R (AFS-9833,³⁴) or rat IgG (Sigma).

Histochemistry, immunohistochemistry and immunofluorescence

P14 mouse pups were perfused with periodate-lysine-2% paraformaldehyde-0.05% glutaraldehyde, pH 7.4 (PLPG),³⁵ their intestines from the anus to stomach removed and opened longitudinally by incision along the length of the intestine, the contents removed by rinsing in PBS and the intestines fixed in PLPG overnight, prior to immersion in 70% ethanol. Paraffin embedding of the tissues was arranged such that tissue orientation could be determined. Sections were treated with periodic acid, then stained in Schiff's reagent (0.5% pararosaniline, 1% sodium metabisulfite; PAS staining) and counterstained with hematoxylin. Sections were also stained with Alcian Blue. The mouse anti-PCNA antibody, PC-10 (1:800) (Dako, #M0879) was used to identify PCNA followed by processing with the Dako Envision + mouse detection kit. Cryptdin 2 (1:400) and CRS4C (1:800) Immunohistochemistry was performed according to prescribed protocols,³⁶ and developed using the ImmPress anti-rabbit detection kit (Vector Laboratories, CA, #MP-7401). Lysozyme was detected with rabbit anti-human lysozyme (1:400) (Dako, #A0099). For the immunofluorescence studies, mice were perfused with ice-cold 4% paraformaldehyde (PFA), pH 7.2 and the dissected and cleaned tissue fragments further fixed (PFA, overnight, 4°C), incubated successively in 15%, then 30% sucrose in phosphate buffered saline (PBS) (8h, 4°C each) and frozen in OCT. Sections (30 µm) were incubated with rabbit anti-human lysozyme (1/100) and affinity purified goat anti-mouse CSF-1R peptide polyclonal antibodies,³⁷ followed by incubation with FITC-conjugated donkey anti-rabbit IgG (1/200) and TRITC-conjugated donkey anti-goat IgG (1/200) (Southern Biotech) and photography using a Olympus Bx51 upright microscope. For localization of β -galactosidase, tissues from mice perfused with PBS were fixed (1.5% PFA, 30% sucrose in PBS, pH 7.2, 8hr, 4°C), frozen in OCT and 10 µm sections stained with X-gal (overnight, 32°C) as described.³⁸ Cell counting: longitudinal sections, blinded for genotype, were identified at 100X magnification, at which the lumen could be seen to traverse the crypt plus villus and then counted at 400X magnification. At least 40 crypts (plus villi) were examined per genotype with 3–5 mice per genotype. Data were analysed using the ANOVA program provided by the Origin 7.5R statistical package.

Quantitative RT-PCR analysis of crypt epithelium RNA

SI crypt epithelium was prepared and its purity confirmed as described.³⁹ Real-time RT-PCR reactions were conducted on genomic DNA-depleted RNA using the Bio-Rad iQ-5 i-cycler system (Bio-Rad Laboratories, CA) and the appropriate primers (Supplementary Table 1). Gene expression was normalized to β 2-microglobulin.

RESULTS

Disrupted morphology in the SIs of *Csf1^{op/op}* and *Csf1r^{-/-}* mice

2-week-old *Csf1^{op/op}* and *Csf1r^{-/-}/Csf1r^{-/-}* (*Csf1r^{-/-}*) mutant FVB/NJ pups were smaller than their WT littermates, as described previously^{30, 40} and their SIs were shorter (Supplementary Fig. 1 online). Tissue sections stained with the neutral mucin stain, periodic acid-Schiff reagent (PAS) (Fig. 1A) or the acid mucin stain, Alcian Blue (AB) (Supplementary Fig. 2 online) revealed an aberrant goblet cell staining in the SI villi of mutant mice compared to WT littermates. The mucin-stained vacuoles in mutant villi were approximately double the diameter of those in WT villi and there was a 3.6-fold increase in the average number of PAS positive cells and a 2.3-fold increase in average AB positive cells per crypt and villus compared with WT (PAS: WT, 3.34 ± 0.29 ; *Csf1^{op/op}*, 10.63 ± 1.33 ; *Csf1r^{-/-}*, 11.35 ± 0.33 ; 50 crypts, mean $\pm$ SEM and AB: Supplementary Fig. 2 online). The villus tips were highly vacuolated with vacuoles that were mostly devoid of mucin, resembling the morphology of the villi reported in patients with malabsorption and lipid-engorgement disorders.⁴¹

The appearance of the mutant villi was also abnormal, with spherical enterocyte nuclei that were often more darkly basophilic than the ordered and uniformly-stained columnar nuclei in WT SI, particularly at the villus tip (Fig. 1B). The average number of cells per crypt (Fig. 1C) and per villus (Fig. 1D) was significantly reduced in both mutants. The SIs of both mutants also had reduced numbers of the rarer Chromogranin A-positive enteroendocrine cells (Supplementary Fig. 3 online), concordant with the overall reduction in enterocytes in *Csf1^{op/op}* and *Csf1r^{-/-}* mice. While there was no significant difference between the two mutant mice in these parameters (Fig. 1, C and D; Supplementary Fig. 3 online), the degree of architectural disorganization was more severe in *Csf1r^{-/-}* than in the *Csf1^{op/op}* (Fig. 1, A and B; Supplementary Fig. 2 online).

Severe depletion of mature PCs in the SIs of CSF-1- and CSF-1R-deficient mice

Mouse PCs appear after birth when the intestinal crypts are formed.¹⁷ PAS staining of the PC granules⁴² indicated that PCs were severely depleted in the mutant mouse SI crypts (data not shown). To better assess the status of PC in the mutant mice, tissue sections were stained for lysozyme,⁴³ which showed that both mutant mice had very few PCs (Fig. 2, A–C). The number of lysozyme⁺ cells per crypt cross-section indicated a significant deficit in PC number that was more severe in the *Csf1r^{-/-}* mice than the *Csf1^{op/op}* mice (Fig. 2, D–F). Similar results were obtained with the PC markers Cryptdin 2 and CRS4C (Supplementary Figs. 4 & 5 online). The high power images also confirmed the presence of macrophage-like cells in the lamina propria of the WT²⁵, but rarely in mutant mice (Fig. 2, E and F). The *Csf1r^{-/-}* (FVB/NJ) mice do not survive beyond 4 weeks. However, a reduction in both PC and macrophages persisted in adult *Csf1^{op/op}* mice (Supplementary Fig. 6 online).

To independently evaluate the CSF-1R dependence of PC development, we subcutaneously injected neutralizing anti-CSF-1R antibody^{33, 34} or control immunoglobulin into WT mice from 3.5 days of age every third day until day 12.5. Mice were sacrificed at day 14 and their SIs stained for PC with anti-lysozyme antibody (Fig. 3, A and B). Consistent with the result obtained with *Csf1r^{-/-}* mice (Fig. 2 D), the SIs of mice treated with anti-CSF-1R antibody versus control IgG had decreased numbers of PC (IgG-treated, 46.8 ± 7.3 ; CSF-1R antibody-treated, 19.0 ± 4.6 ; mean $\pm$ SD, counts/6 fields, $p \geq 0.005$). Together, these experiments demonstrate that CSF-1 and the CSF-1R play a major role in the development and maintenance of PC.

Reduced proliferating progenitor cells in the SIs of *Csf1^{op/op}* and *Csf1r^{-/-}* mice

The proliferating progenitor cells that give rise to the epithelial cells of the SI (including the PC) can be identified immunohistochemically by staining for proliferating cell nuclear antigen (PCNA). Because of the smaller villus length and the reduced cell number per unit length of the crypts and villi, we examined PCNA-stained SI sections to determine the effect of the mutations on the number of proliferating progenitor cells. The distribution and extent of PCNA staining was clearly and significantly affected in the SIs of both mutant mice (Fig. 4, A–C). Apart from an overall reduction in the average number of PCNA⁺ cells (Fig. 4D), they were increased in frequency at the crypt base in mutant versus WT SIs at sites in which PCs are found normally. To further elucidate this proliferative defect, we isolated SI crypt epithelium and quantified the expression of a CSF-1R target gene, *cyclin D1*⁴⁴ and an intestinal stem cell marker, *Lgr5*, via real-time RT-PCR. Similar to the reduction of PCNA⁺ cell (Fig. 4D), we found a significantly decreased expression of both genes in the SI crypt of *Csf1r^{-/-}* versus WT mice (Fig 4 E).

PCs and SI macrophages express the CSF-1R

To determine whether the decreased numbers of PCs, enterocytes and enteroendocrine cells in *Csf1^{op/op}* and *Csf1r^{-/-}* mice were possibly due to the direct regulation by CSF-1, we stained

WT sections using a specific (Supplementary Fig. 7 online), anti-CSF-1R peptide antibody. There was strong CSF-1R staining of macrophages in the lamina propria and below the crypt base, as previously reported²⁵ and of PC, that co-localized with anti-lysozyme antibody staining (Fig. 5, A–C), but no staining of other SI cell types. As reported previously for lysozyme,⁴⁵ the PC staining was within and outside granules (Supplementary Fig. 8 online).

CSF-1-synthesizing cells are in close proximity to PCs

Since PC express the CSF-1R, it was likely that there were CSF-1-synthesizing cells nearby. To detect them, a transgenic mouse line (TgZ9) that expresses the beta-galactosidase gene (β -gal) under the control of the *Csf1* promoter and first intron was used in which nuclear expression of the β -gal reporter is driven in a cellular pattern recapitulating expression of the endogenous *Csf1* gene.³² Histochemical staining of β -gal in the SIs of *TgZ9/TgZ9* and non-transgenic control mice showed that *CSF-1* promoter activity was absent in control tissue (Fig. 6A), but present in cells that are in close proximity to the crypt base in the region of PCs in the SIs of transgenic mice (Fig. 6, B–D).

PCs are locally regulated by cell-surface CSF-1 isoform-expressing cells

The close proximity of the CSF-1-synthesizing cells to PC suggested that the PC might be locally regulated by them. We therefore examined *Csf1^{op/op}*; *TgCS5/+* mice that exclusively express the cell surface isoform of CSF-1 (csCSF-1) driven by the same *CSF-1* promoter and first intron sequence as used in TgZ9, and that fail to express detectable circulating CSF-1.⁴⁶ Rescue of the proliferation, crypt and villus defects of *Csf1^{op/op}* mice by expression of csCSF-1 was essentially complete (Fig. 7A). Importantly, normal PC numbers were also restored in mice exclusively expressing csCSF-1 (Fig. 7B) as were lamina propria macrophages (Supplementary Fig. 9 online). These results demonstrate that local expression of csCSF-1 is sufficient for PC development and/or maintenance.

DISCUSSION

PCs play a critical role in the innate immune response to bacteria.^{16–18} CSF-1 regulation of macrophages,²⁵ trophoblastic cells,⁴⁷ and Langerhans cells³¹ is important for innate immunity in many tissues.⁴⁸ We have shown that both PCs and macrophages in the SI express high levels of the CSF-1R and that, as for macrophages,²⁵ the development of PCs is largely dependent on the CSF-1R. Furthermore, CSF-1-synthesizing cells are found in close proximity to PCs and normal regulation of PC development was observed when, in the absence of circulating CSF-1, these cells exclusively expressed membrane-spanning csCSF-1. These results indicate that CSF-1 locally regulates PC development.

Apart from the PC and macrophage deficiency, other changes contributed to the disruption of SI architecture observed in the *Csf1^{op/op}* and/or *Csf1r^{-/-}* mice. First, there was a reduction in the major epithelial cell population, the villus columnar cells. Second, there was an increase in both the number of the goblet cells and their cytoplasmic volume. Third, there was a reduction in Chromogranin A-positive enteroendocrine cells. Fourth, consistent with the overall reduction in mature SI epithelial cells, the number of proliferating progenitor cells in the crypt was significantly reduced. Finally, concordant with the decrease in proliferating progenitor cells, we observed a reduction in the expression of *cyclin D1* and *Lgr5* in the SI crypts of *Csf1r^{-/-}* mice.

The increase in the number of goblet cells may be a consequence of CSF-1R-regulated PC development. Previous studies showed that PCs and goblet cells share an intermediate cell precursor with shared features of both cell types.^{14, 49} When PC differentiation of these or other precursors was inhibited by transgenic, PC-specific expression of SV40 T antigen, the

survival, proliferation and differentiation of the intermediate cell to mature goblet cells in the upper crypt and lower half of the villus was increased.⁴⁹ Thus, the increase in the number of goblet cells we observed when PC development was inhibited by removal of the CSF-1R, is consistent with a default fate of the intermediate cell precursor to goblet cell differentiation.

The reduction in non-PC epithelial cells of the columnar/enterocyte and enteroendocrine lineages, together with the overall decrease in progenitor cell proliferation and reduction in expression of the intestinal stem cell marker, Lgr5¹⁵, indicates that CSF-1R signaling controls progenitor cell proliferation and may regulate lineage determination at the stem/progenitor cell level. However, CSF-1R expression was detected only in PC and interstitial macrophages (Fig. 5 and data not shown) suggesting that this regulation of the CSF-1R-negative stem/progenitor cells is indirect. It is unlikely that the deficits we have observed in the *Csf1r*^{-/-} mice are secondary to the loss of PC, since transgenic ablation of PCs had no qualitative or quantitative effects on the other three SI cell lineages or on the stem cell compartment.⁴⁹ On the other hand, given growing evidence for the role of CSF-1 and macrophages in the development⁵⁰ and regeneration⁵¹ of epithelia and in the regulation of malignant epithelial cells,⁵² it is possible that the interstitial macrophages of the lamina propria contribute to the regulation of progenitor cell expansion and commitment and that their loss in CSF-1/CSF-1R deficiency leads to the deficits in these SI lineages. Indeed, while PC expression of the CSF-1R, their close proximity to CSF-1-expressing cells and their normal development in mice exclusively expressing csCSF-1 are consistent with direct juxtacrine/paracrine regulation of PC by CSF-1, our experiments do not formally exclude indirect regulation of PC development by the lamina propria macrophages, which are also co-ordinately rescued by csCSF-1 expression.

The demonstration that locally produced CSF-1 regulates PC development is consistent with studies suggesting that terminal differentiation of the SI epithelial lineages is largely cell non-autonomous and apparently dependent on signals from specific positions along the crypt-villus axis.⁵³ Previous studies have implicated the WNT signaling pathway transduced through β -catenin/TCF4, in maintaining the undifferentiated state of intestinal epithelial progenitor cells,^{54, 55} as well as PC specification⁵⁶. Furthermore, loss of the *adenomatous polyposis coli* (*Apc*) gene, which normally negatively regulates WNT signalling,⁵ perturbed differentiation along the enterocyte, goblet and enteroendocrine lineages, while promoting partial differentiation to the PC lineage through β -catenin/TCF4 regulated transcription of specific markers of PC.⁵⁷ Also, conditional removal of the common Notch pathway transcription factor, CSL/RBP-J decreases proliferative cells and increases goblet cells.⁵⁸ It will be of interest to determine how regulation by the CSF-1R is related to WNT- and Notch- regulated differentiation.

The CSF-1R could directly or indirectly mediate commitment to the PC lineage, support the survival of committed cells, or regulate aspects of PC differentiation. While CSF-1 plays a major role in PC development, the effects of CSF-1R deficiency were more severe than those of CSF-1 deficiency (Fig. 2 D). It has been suggested that this difference, observed for other phenotypes, is due to the existence of another CSF-1R ligand.²⁹ Indeed, it is possible that regulation by the novel CSF-1R ligand, interleukin-34,⁵⁹ is responsible. In addition, the small residual PC development in *Csf1r*^{-/-} mice suggests a secondary role of a non-CSF-1R ligand.

Independent of its regulation of PC development, CSF-1 could also regulate PC function. CSF-1 is known to prime the macrophage response to bacterial lipopolysaccharide⁶⁰ and lipopolysaccharide enhances CSF-1 synthesis and release from CSF-1-synthesizing cells.⁶¹ If CSF-1 has a similar role in regulating the PC response to bacteria, increased bacteria-induced local expression of CSF-1 in the SI can be expected to contribute to regulation of the anti-bacterial response of PC. The CSF-1-synthesizing cell type in the crypt region is not known.

A possible candidate is the subepithelial myofibroblast,⁶² a morphological hybrid of a smooth muscle cell and a fibroblast, that continuously migrate upward from the crypt base.⁶³

The demonstration that CSF-1 is required for PC genesis and normal SI development has important implications for our understanding of intestinal development and disease. Accumulating evidence suggests that defective PC differentiation and function underpins intestinal disease.^{16, 18, 64–67} For example, diminished defensin production has recently been associated with Crohn's disease and ulcerative colitis.⁶⁶ PCs also secrete the anti-bacterial, pro-inflammatory phospholipase A2, expression of which also suppresses the development of intestinal adenomas in *APC^{min}* mutant mice¹⁶ as well as matrilysin, a protease essential for the correct processing of the multiple defensin precursor proteins and for maximum tumor invasion.⁶⁸ In addition, relevant to our findings, administration of anti-CSF-1 antibody inhibits the development of dextran sulfate sodium-induced colitis in mice.⁶⁹ Taken together, these data indicate a regulatory role of CSF-1 in PC development and the need for further investigation of the biological roles of CSF-1 in the intestine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used in this paper

AB	alcian blue
CSF-1	colony stimulating factor
CSF-1R	colony stimulating factor receptor
PAS	periodic acid Schiff
PC	Paneth cell
PCNA	proliferating cell nuclear antigen
SI	small intestine

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Figure 1. Abnormal SI crypts and villi in two-week-old *Csf1r*^{-/-} and *Csf1*^{op/op} mice

(A) Longitudinal sections stained for neutral mucins (PAS-stain) reveal intense staining of goblet cells in the SIs of the mutant mice. (B) Higher power images of the regions boxed in (A) reveal the enlarged mucin-containing deposits in goblet cells (arrows). In addition, the less ordered organization of nuclei in the mutant villi is also evident. Bars = 50 μ m. (C) Numbers of cells/crypt length and (D) number of cells/villus length (mean $\pm$ SEM; n=3–5/genotype, ANOVA P-values shown).

Figure 2. Lysozyme staining reveals a dramatic reduction of PCs in the SI crypts of two-week-old *Csf1r*^{-/-} and *Csf1*^{op/op} mice

Low power images of lysozyme⁺ Paneth cells in the SIs of (A) WT, (B) *Csf1r*^{-/-} and (C) *Csf1*^{op/op} mice. (D) Quantitation of the number of lysozyme⁺ cells/crypt section (mean ± SEM, n = 3, ANOVA P-values shown), revealed significant differences the number of PCs at the crypt base (grey arrow in E) between both mutant and WT, as well as between mutants. (E) Higher power images of WT crypts showing the presence of lysozyme⁺ cells within the lamina propria indicative of macrophages (black arrow). (F) Only background lysozyme staining is evident in the *Csf1r*^{-/-} SI. White arrow indicates goblet cell with enlarged mucin body. Bar = 50µm.

Figure 3. Treatment of WT mice with anti-CSF-1R antibody inhibits development of PCs and villus macrophages

Newborn mice were subcutaneously injected with (A) control IgG or (B) anti-CSF-1R antibody for 14 days prior to fixation and sectioning of the SIs and staining with anti-lysozyme antibody and DAPI. Bar = 60μm.

Figure 4. SI crypts of two-week-old *Csf1r*^{-/-} and *Csf1*^{op/op} mice display hypoproliferative defects PCNA staining of (A) WT (B) *Csf1r*^{-/-} and (C) *Csf1*^{op/op} SI reveals proliferation deficits in the mutant crypts. (D) Reduced numbers of PCNA⁺ nuclei/*Csf1r*^{-/-} or *Csf1*^{op/op} crypt compared with WT littermate crypts (mean ± SEM; n=3/genotype, 25 crypt/villi per mouse counted, ANOVA P-values shown). Bar = 50μm. (E) Quantitative RT-PCR analysis of *Csf1r*, *cyclin D1* and *Lgr5* in crypts of *Csf1r*^{-/-} and WT littermates (mean ± SEM; ND = no data, n=3, ANOVA P-values shown).

Figure 5. PCs and SI macrophages express the CSF-1R

Immunofluorescence micrography showing: (A) CSF-1R staining (red) of cells within the crypt (PC), below the crypt and within interstitial regions of the villi (macrophages), (B) staining for lysozyme (green) and (C) merged images, showing co-localization of the CSF-1R and lysozyme staining. P, PCs; M, macrophages. Nuclei counterstained with DAPI (blue). Bar = 30µm.

Figure 6. CSF-1 is expressed in close proximity to PCs

Histochemical staining for beta-galactosidase in the SIs of two-week-old (A) control WT and (B–D) *TgZ9/TgZ9* mice. (B), Low power and (C) and (D), high power fields, showing the close proximity of β -gal staining (blue, nuclear, arrowed) to the PCs (P). Note: There is spill-over of nuclear beta-galactosidase expression into cytoplasm in some cells.

Figure 7. Expression of the membrane-spanning, cell-surface isoform of CSF-1 is sufficient to rescue the SI defects of two-week-old *Csf1^{op/op}* mice
(A) Rescue of proliferating (PCNA), crypt and villus cells. (B) Rescue of PCs. Bar = 50 μ m.