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Involvement of Ly6C, 4-1BB, and KLRG1 in the activation of lamina propria lymphocytes in the small intestine of sanroque mice

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Abstract

Roquin is an E3 ligase that regulates mRNA stability. Mice with a mutation in the *Rc3h1* gene and Roquin protein, referred to as Roquin$^{san/san}$ or sanroque mice, develop broad-spectrum chronic inflammatory conditions and autoimmune pathologies. Our laboratory recently reported that sanroque mice also develop extensive inflammation that is localized in the small intestine but is rare in the colon. Here, we demonstrate that small intestinal intraepithelial lymphocytes (IELs) are present in the epithelium of sanroque mice but that cell recoverability is low using standard extraction techniques even though lamina propria lymphocytes (LPLs) can be recovered in normal numbers. In studies aimed at characterizing T cell costimulatory markers and activation molecules on LPLs in sanroque mice, we identified Ly6C and 4-1BB (CD137) as being expressed at elevated levels on sanroque small intestinal LPLs, and we show that both of those subsets, in conjunction with cells expressing the KLRG1 T cell activation molecule, are sources of IL-17A, IFN-γ, and TNFα. TNFα was primarily produced by 4-1BB+, KLRG1- cells, but was also made by some 4-1BB-, KLRG1- cells, and 4-1BB-, KLRG1+ cells. These findings collectively suggest that the small intestinal inflammatory response in sanroque mice is driven, at least in part, by LPL activation through Ly6C and 4-1BB signaling, and they provide further evidence in support of using the sanroque mouse as an animal model of chronic small intestinal inflammation.

Keywords

Costimulation; Inflammation; Intestine; Proinflammatory cytokine; Roquin

1. Introduction

Roquin is an E3 ubiquitin ligase that binds to target mRNAs and regulates post-translational protein expression [1,2,3,4]. A mutation in the *Rc3h1* gene for roquin of C57BL/6 mice made by treating animals with *N*-ethyl-*N*-nitrosourea resulted in a methionine to arginine

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Conflict of Interest

The authors declare no conflict of interest.

substitution at position 199 of the roquin protein. Those animals, referred to as Roquin$^{san/san}$ or sanroque mice [5], have extensive autoimmune and chronic inflammatory responses that include antibodies to double-stranded DNA, IgG immune complexes, glomerulonephritis, anemia, necrotizing hepatitis, splenomegaly and lymphadenopathy, and thrombocytopenia and plasmocytosis [5]. Germinal center dysorganization with large numbers of follicular T helper cells are a hallmark of the immunological perturbation in sanroque mice [5,6]. Additionally, sanroque mice have increased expression of inducible costimulator (ICOS), regulated upon activation, normal T-cell expressed (RANTES), CXCR5, and Programmed cell Death-1 (PD-1) [7,8,9].

Studies in our laboratory demonstrated that sanroque mice develop chronic inflammation at high penetrance (91.3% after 8 wk of age) that is manifest throughout the small intestine, but not in the colon [8]. Similar changes occur in mice in which the *Rc3h1* gene has been deleted [8]. Mesenteric lymph node T cells in sanroque mice express ICOS, OX40, and the KLRG1 activation molecules [8]. Interestingly, although we have been able to recover LPLs from sanroque mice, IELs have been consistently difficult to recover using standard extraction techniques, an observation that is addressed in the present study.

Documentation that intestinal inflammation in sanroque mice is driven by leukocytes and is not a systemic defect was shown in studies in which bone marrow from *Rc3h1*-deficient mice was adoptively transferred to irradiated control mice [10], which resulted in chronic small intestinal inflammation similar to that of sanroque mice. Moreover, lamina propria lymphocytes (LPLs) in mice that receive *Rc3h1*-deficient bone marrow had elevated levels of IL-17A, IFN-γ, and TNFα, although the specific effector subsets responsible for the production of those cytokines was not determined. Those cells expressed an effector-memory T cell phenotype [11].

To further understand the basis for the inflammatory response in the small intestine of sanroque mice, we have examined the functional involvement of three activation molecules, Ly6C, 4-1BB, and KLRG1, with regard to proinflammatory cytokine synthesis. Our findings indicate that all three molecules contribute, either collectively or individually, to that response. These findings have implications for understanding chronic small intestinal inflammation in a natural setting that does not require the administration of toxic substances or by transfer of effector T cells.

2. Materials and methods

2.1. Animals

Mating pairs of Roquin$^{san/+}$ mice were obtained from the Mutant Mouse Regional Resource Center at the University of California, Davis, CA. Heterozygous Roquin$^{san/+}$ mice were intercrossed to produce homozygous Roquin$^{san/san}$ (sanroque) mice. A mutagenically-separated PCR protocol [8] was used to determine zygosity in which a PCR product of 190 bp indicated the wild type allele while a product of approximately 215 bp denoted the presence of the Roquin san mutation; both products were present in Roquin$^{san/+}$ mice. Experimental procedures using animals were approved by the Institutional Animal Care and Use Committee permit No. HSC-AWC-12-039.

2.2. Cell isolation and enumeration, staining, and flow cytometry

IELs and LPLs were isolated using standard procedures for disruption of the epithelium, and digestion of the basement membrane using collagenase, respectively, as described by our laboratory [8,10]. Cells were filtered through nylon wool, collected, and centrifuged at 400g through a 40%/70% Percoll gradient. Viable cells were collected from the interface.

For flow cytometry, cells were stained using appropriate combinations of the following antibodies: PE- and FITC-anti-CD3ε (145-2C11); PE- and FITC-anti-CD4 (GK1.5); PE- and FITC-anti-CD8α (53.6); FITC-anti-CD45.2 LCA (104); PE-anti-4-1BB (17B5); APC-KLRG1 (2F1); FITC-anti-IFNγ (YMG1.2); FITC-anti-IL17 (17B7); FITC-anti-TNFα (MP6-XT22); streptavidin APC (17-5317-82) (eBioscience, San Diego, all reagents). Biotin-anti-Ly6C (HK1.4, Southern Biotech, Birmingham, AL). Control staining was done with labeled IgG-matched antibodies (eBioscience and Southern Biotechnology). Surface and intracellular staining was done as previously described [8,12]. Fluorescence was assessed using a FACSCalibur flow cytometer with CellQuest software (BD Bioscience, San Jose, CA).

Tissue staining for CD3 was done using representative formalin-fixed paraffin-embedded tissue sections after deparaffinization, rehydration, and antigen retrieval in Target Antigen Retrieval Solution (Target Retrieval solution 10x Concentrate, Dako, Santa Cruz, CA) according to the manufacturer's instructions. Endogenous peroxidase activity was quenched by incubating the tissue section with 3% H_2O_2 in methanol for 30 min. Mouse-on-mouse non-specific binding sites were blocked by incubating the sections in MOM mouse Ig blocking solution (Vector Laboratories, Burlingame, CA) for 1 hr. A second non-specific blocking step was performed using 15 μL/mL horse serum diluted in Tris-HCL with 1% BSA for 5 min. Tissues were reacted with rabbit anti-mouse CD3ε antibody (SP7, ThermoFisher Scientific, Waltham, MA), followed by biotinylated linking to a peroxidase (LSAB2 System-RP) (Dako) according to the manufacturer's instructions. Immunoperoxidase reactive sites in tissue sections were visualized using a DAB substrate kit (Vector Laboratories) for chromogenic detection.

2.3. Enzyme-linked assays (EIAs)

Sanroque LPLs were stimulated using plate-bound anti-CD3 antibody, or anti-CD3 antibody plus anti-Ly6C for 24 hrs. Cell-free supernatants were assayed for secreted IL-17, IFN-γ, and TNFα using commercial Ready-SET-Go EIA kits (eBioscience).

3. Results

3.1. Low recoverability of small intestinal IELs but not LPLs from sanroque mice

During the course of our studies into the inflammatory response in the small intestine of sanroque mice, we have been consistently unable to recover significant numbers of IELs from the small intestine, although LPLs were recovered at normal levels relative to WT mice. To explore this in greater detail, we have conducted as series of IEL isolates from sanroque and WT mice. Out of seven isolates from sanroque mice, no IELs were recovered from five animals; two mice had extremely low numbers ($<10^6$) of cells, collectively

averaging ~1 × 10^5 cells/animal (Fig. 1A). In contrast, 15.2 ±1.5 × 10^6 IELs were recovered from nine WT mice (Fig. 1A). Unlike IELs, LPLs were present in equivalent numbers in sanroque and WT mice (Fig. 1A). Based on immunocytochemical staining of small intestinal tissues sections, CD3+ IELs were present in equivalent numbers in both sanroque and WT mice (Fig. 1B–E). Although the reason for the low recoverability of IELs from sanroque mice is unclear, it is possible that cell adhesion molecules and their ligands are upregulated on IELs and epithelial cells in sanroque mice, thus causing IELs to be retained in the epithelial layer. Considerably more work will be needed to address this.

3.2. Ly6C upregulation on LPLs of sanroque mice is linked to the expression of three proinflammatory cytokines

Due to the inability to recover IELs from sanroque mice and the presence of sufficient numbers of LPLs, experiments were conducted to assess the expression of activation markers on LPLs. Our previous studies demonstrated that CD43 [13,14], Ly6C (CD59) [15], CD69 [15], OX40 (CD134) [16], 4-1BB (CD137) [17], By55 (CD160) [18], ICOS (CD278) [12,19], and IL-18 and the IL-18R [20] are expressed in the small intestinal mucosa. Two of the most interesting of those, and the least studied to the present, are Ly6C and 4-1BB. Work in our laboratory demonstrated that Ly6C is expressed on a small proportion of normal small intestinal IELs [15], and that activation of IELs via CD3 triggering resulted in upregulation of Ly6C, as well as other costimulatory molecules such as OX40 [21]. Interestingly, trigger via CD43 independent of CD3 stimulation also lead to a high percent of IELs expressing Ly6C [21], thus suggesting that multiple routes of activation may influence the expression of Ly6C on small intestinal T cells.

Based on those observations [15,21,22], we were interested in examining the role of Ly6C on LPLs in the small intestine of sanroque mice. To that end, we characterized the expression of Ly6C on total leukocytes (CD45+), total T cells (CD3+), CD4+ T cells, and CD8+ LPLs in WT (Fig. 2A) and sanroque (Fig. 2B) mice, which indicated a marked increase in Ly6C expression on all cell subsets of LPLs in sanroque mice compared to WT mice. This included an increase in both the overall proportion of Ly6C+ cells (Fig. 2C), but also the density of surface expression of Ly6C as determined by the mean fluorescence intensity (MFI) (Fig. 2D). LPLs isolated from sanroque and WT mice were stained for expression of Ly6C and intracellular IL-17A, IFN-γ, and TNFα. All three proinflammatory cytokines were expressed at higher levels in Ly6C+ cells from sanroque mice (Fig. 2E). Additionally, LPLs were cultured with anti-CD3 antibody ± anti-Ly6C costimulation. Twenty-four hr later cells were stained for secretion of IL-17, IFNγ, and TNFα by EIA. Ly6C costimulation resulted in elevated responses of all three cytokines (Fig. 2F). These findings point to a direct role for Ly6C on sanroque T cells in driving the small intestinal inflammatory response.

3.3. 4-1BB is expressed on CD4+ LPLs in sanroque mice, and 4-1BB+ cells co-express KLRG1.

4-1BB is expressed at very low levels on normal small intestinal T cells, both CD4+ (Fig. 3A) and CD8α+ (Fig. 3B) cells, but is rapidly acquired during activation as shown in cultures of non-stimulated (Fig. 3C) vs. CD3-stimulated T cells (Fig. 3D). LPLs isolated

from sanroque mice had more 4-1BB+ cells (Fig. 3, bottom row) compared to WT animals (Fig. 3, middle row). The majority of the 4-1BB+ cells were CD4+, and about half the 4-1BB+ cells expressed KLRG1 (Fig. 3, bottom row).

3.4. A population of 4-1BB+ KLRG1- cells is the dominant source of TNFα by sanroque LPLs.

To further refine our understand the source of the IL-17A, IFNγ, and TNFα proinflammatory cytokines on activated LPLs from sanroque mice, LPLs were stimulated in vitro with plate bound anti-CD3 antibody. Non-stimulated cells had undetectable intracellular cytokine production (data not shown). The most significant observation from these studies was the high proportion of cells (12.7%) that produced TNFα among the 4-1BB+ KLRG1- subset (Fig. 4C). Additionally, TNFα was made by 3.3% of the 4-1BB- KLRG1+ cells, and 5.9% of the 4-1BB- KLRG1- cells. (Fig. 4C). In contrast, neither 4-1BB + nor KLRG1+ were producers of IL-17A (Fig. 4A) or IFNγ (Fig. 4B), although some 4-1BB- KLRG1- cells produced IL-17A and IFNγ.

4. Discussion

At present, few animal models exist that resemble the pathological features of Crohn's disease in the small intestine. We recently reported that sanroque mice develop chronic small intestinal inflammation in all regions of the small intestine (duodenum, jejunum, and ileum) as well as in the cecum, but do not develop colonic inflammation [8,10]. During the course of those studies, there was extremely low recoverability of IELs, but not LPLs, from sanroque mice. There were two possible explanations for this. The first is that the small intestinal epithelium is devoid of IELs in sanroque mice. The second is that although the cells are present, they are retained in the epithelium more effectively than in WT mice. Both of those would have functional explanations for the intestinal inflammatory response in sanroque mice. In the present study, in a series of cell isolates from the small intestine, we confirmed our prior observations of low recoverability. Importantly, however, by immunocytochemical staining we observed that IELs were present in the epithelium of sanroque mice, thus pointing to a situation in which IELs are not released from the epithelium. Although the reason for this is unclear at this point, there are several potential causes. It is conceivable that adhesion molecules and their ligands are upregulated and expressed in the small intestinal epithelium in sanroque mice, thereby promoting the inflammatory response. This could include changes in P-selectin glycoprotein ligand-1, P-selectin and/or E-selectin, α4β7, CCR9, CCL25, LFA-1, very late antigen-4 of epithelial cells, MAdCAM-1, among others, all of which have been shown to be expressed at varying levels in the intestinal epithelium [23,24,25,26,27,28]. Of these, we have demonstrated that CCL25 is elevated in the ileum of sanroque mice [8].

Additionally, we were interested in better understanding the basis for the inflammatory response in the small intestine of sanroque mice, primarily through the analyses and functional characterization of two T cells costimulatory molecules, Ly6C and 4-1BB. Ly6C has been shown to enhance the lytic activity and cytokine production of CD8+ T cells [29,30,31]. As reported here, Ly6C was expressed on both CD4+ and CD8+ LPLs in

sanroque mice, with more than half of the CD8+ cells being Ly6C+. The upregulated expression of Ly6C and the synthesis of IL-17A, IFN-γ, and TNFα by Ly6C+ LPLs identifies Ly6C as an activator of inflammatory responses in the intestinal mucosa of sanroque mice.

The expression of 4-1BB differed from that of Ly6C in that it was primarily expressed on CD4+ T cells. Interaction of 4-1BB with its ligand 4-1BBL on antigen presenting cells leads to T cells proliferation and cytokine production, which can be blocked by anti-4-1BB antibody [32,33]. Moreover, human ileal and colonic tissues from Crohn's disease patients have been shown to express higher levels of 4-1BB [34]. Our findings in sanroque mice parallel that observation. Additionally, we demonstrated that the majority of 4-1BB+ cells expressed the T cell activation molecule, KLRG1, thereby further defining the T cell activation phenotype in the small intestine of sanroque mice within a subset primarily consisting of 4-1BB+, KLRG1- cells, though some TNFα was also produced by 4-1BB-, KLRG-1- cells and 4-1BB-, KLRG1 cells.

In summary, this study has provided new information into the mechanisms through which chronic inflammatory response is maintained in the small intestine of sanroque mice. Because few animal models of small intestinal Crohn's-like disease exist, this work lends additional credence to the utility of using sanroque mice for this purpose.

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Abbreviations:

ICOS	inducible costimulator
IELs	intraepithelial lymphocytes
KLRG1	killer cell lectin-like receptor subfamily G member 1
LPLs	lamina propria lymphocytes
LCA	leukocyte common antigen
PD-1	programmed cell death-1
RANTES	regulated upon activation normal T cell expressed

References

[1]. Athanasopoulos V, Barker A, Yu D, Tan AH, Srivastava M, Contreras N, Wang J, Lam KP, Brown SH, Goodnow CC, Dixon NE, Leedman PJ, Saint R, Vinuesa CG, The ROQUIN family of proteins localizes to stress granules via the ROQ domain and binds target mRNAs, FEBS J 277 (2010) 2109–2127. [PubMed: 20412057]

[2]. Kulkarni M, Ozgur S, Stoecklin G, On track with P-bodies, Biochem Soc Trans 38 (2010) 242–251. [PubMed: 20074068]

[3]. Glasmacher E, Hoefig KP, Vogel KU, Rath N, Du L, Wolf C, Kremmer E, Wang X, Heissmeyer V, Roquin binds inducible costimulator mRNA and effectors of mRNA decay to induce microRNA-independent post-transcriptional repression, Nat Immunol 11 (2010) 725–733. [PubMed: 20639877]

[4]. Mino T, Murakawa Y, Fukao A, Vandenbon A, Wessels HH, Ori D, Uehata T, Tartey S, Akira S, Suzuki Y, Vinuesa CG, Ohler U, Standley DM, Landthaler M, Fujiwara T, Takeuchi O, Regnase-1 and Roquin Regulate a Common Element in Inflammatory mRNAs by Spatiotemporally Distinct Mechanisms, Cell 161 (2015) 1058–1073. [PubMed: 26000482]

[5]. Vinuesa CG, Cook MC, Angelucci C, Athanasopoulos V, Rui L, Hill KM, Yu D, Domaschenz H, Whittle B, Lambe T, Roberts IS, Copley RR, Bell JI, Cornall RJ, Goodnow CC, A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity, Nature 435 (2005) 452–458. [PubMed: 15917799]

[6]. Linterman MA, Rigby RJ, Wong RK, Yu D, Brink R, Cannons JL, Schwartzberg PL, Cook MC, Walters GD, Vinuesa CG, Follicular helper T cells are required for systemic autoimmunity, J Exp Med 206 (2009) 561–576. [PubMed: 19221396]

[7]. Linterman MA, Rigby RJ, Wong R, Silva D, Withers D, Anderson G, Verma NK, Brink R, Hutloff A, Goodnow CC, Vinuesa CG, Roquin differentiates the specialized functions of duplicated T cell costimulatory receptor genes CD28 and ICOS, Immunity 30 (2009) 228–241. [PubMed: 19217324]

[8]. Schaefer JS, Montufar-Solis D, Nakra N, Vigneswaran N, Klein JR, Small intestine inflammation in Roquin-mutant and Roquin-deficient mice, PLoS One 8 (2013) e56436. [PubMed: 23451046]

[9]. Bertossi A, Aichinger M, Sansonetti P, Lech M, Neff F, Pal M, Wunderlich FT, Anders HJ, Klein L, Schmidt-Supprian M, Loss of Roquin induces early death and immune deregulation but not autoimmunity, J Exp Med 208 (2011) 1749–1756. [PubMed: 21844204]

[10]. Montufar-Solis D, Vigneswaran N, Nakra N, Schaefer JS, Klein JR, Hematopoietic not systemic impairment of Roquin expression accounts for intestinal inflammation in Roquin-deficient mice, Sci Rep 4 (2014) 4920. [PubMed: 24815331]

[11]. Voehringer D, Koschella M, Pircher H, Lack of proliferative capacity of human effector and memory T cells expressing killer cell lectinlike receptor G1 (KLRG1), Blood 100 (2002) 3698–3702. [PubMed: 12393723]

[12]. Schaefer JS, Montufar-Solis D, Vigneswaran N, Klein JR, ICOS promotes IL-17 synthesis in colonic intraepithelial lymphocytes in IL-10−/− mice, J Leukoc Biol 87 (2010) 301–308. [PubMed: 19889730]

[13]. Montufar-Solis D, Garza T, Klein JR, Selective upregulation of immune regulatory and effector cytokine synthesis by intestinal intraepithelial lymphocytes following CD43 costimulation, Biochem Biophys Res Commun 338 (2005) 1158–1163. [PubMed: 16246302]

[14]. Wang HC, Montufar-Solis D, Teng BB, Klein JR, Maximum immunobioactivity of murine small intestinal intraepithelial lymphocytes resides in a subpopulation of CD43+ T cells, J Immunol 173 (2004) 6294–6302. [PubMed: 15528368]

[15]. Wang HC, Zhou Q, Dragoo J, Klein JR, Most murine CD8+ intestinal intraepithelial lymphocytes are partially but not fully activated T cells, J Immunol 169 (2002) 4717–4722. [PubMed: 12391179]

[16]. Wang HC, Klein JR, Multiple levels of activation of murine CD8(+) intraepithelial lymphocytes defined by OX40 (CD134) expression: effects on cell-mediated cytotoxicity, IFN-gamma, and IL-10 regulation, J Immunol 167 (2001) 6717–6723. [PubMed: 11739485]

[17]. Zhou Z, Pollok KE, Kim KK, Kim YJ, Kwon BS, Functional analysis of T-cell antigen 4-1BB in activated intestinal intra-epithelial T lymphocytes, Immunol Lett 41 (1994) 177–184. [PubMed: 8002034]

[18]. Anumanthan A, Bensussan A, Boumsell L, Christ AD, Blumberg RS, Voss SD, Patel AT, Robertson MJ, Nadler LM, Freeman GJ, Cloning of BY55, a novel Ig superfamily member expressed on NK cells, CTL, and intestinal intraepithelial lymphocytes, J Immunol 161 (1998) 2780–2790. [PubMed: 9743336]

[19]. Montufar-Solis D, Garza T, Teng BB, Klein JR, Upregulation of ICOS on CD43+ CD4+ murine small intestinal intraepithelial lymphocytes during acute reovirus infection, Biochem Biophys Res Commun 342 (2006) 782–790. [PubMed: 16500623]

[20]. Montufar-Solis D, Wang HC, Klein JR, Stimulatory and costimulatory effects of IL-18 directed to different small intestinal CD43 T cell subsets, J Leukoc Biol 82 (2007) 1166–1173. [PubMed: 17702824]

[21]. Montufar-Solis D, Klein JR, Activation and Costimulation of Intestinal T Cells: Independent and Collaborative Involvement of CD43, OX40, and Ly-6C, Curr Immunol Rev 1 (2005a) 13–20. [PubMed: 19081760]

[22]. de Geus B, HogenEsch H, Kubo RT, Rozing J, Glycosylphosphatidyl inositol-linked membrane protein expression by intestinal intraepithelial lymphocytes, Int Immunol 4 (1992) 899–903. [PubMed: 1358188]

[23]. Luster AD, Alon R, von Andrian UH, Immune cell migration in inflammation: present and future therapeutic targets, Nat Immunol 6 (2005) 1182–1190. [PubMed: 16369557]

[24]. Mora JR, von Andrian UH, Differentiation and homing of IgA-secreting cells, Mucosal Immunol 1 (2008) 96–109. [PubMed: 19079167]

[25]. Sans M, Panes J, Ardite E, Elizalde JI, Arce Y, Elena M, Palacin A, Fernandez-Checa JC, Anderson DC, Lobb R, Pique JM, VCAM-1 and ICAM-1 mediate leukocyte-endothelial cell adhesion in rat experimental colitis, Gastroenterology 116 (1999) 874–883. [PubMed: 10092309]

[26]. Sans M, Salas A, Soriano A, Prats N, Gironella M, Pizcueta P, Elena M, Anderson DC, Pique JM, Panes J, Differential role of selectins in experimental colitis, Gastroenterology 120 (2001) 1162–1172. [PubMed: 11266380]

[27]. Steeber DA, Tang ML, Zhang XQ, Muller W, Wagner N, Tedder TF, Efficient lymphocyte migration across high endothelial venules of mouse Peyer's patches requires overlapping expression of L-selectin and beta7 integrin, J Immunol 161 (1998) 6638–6647. [PubMed: 9862692]

[28]. Pavlick KP, Ostanin DV, Furr KL, Laroux FS, Brown CM, Gray L, Kevil CG, Grisham MB, Role of T-cell-associated lymphocyte function-associated antigen-1 in the pathogenesis of experimental colitis, Int Immunol 18 (2006) 389–398. [PubMed: 16415103]

[29]. Johnson R, Lancki DW, Fitch FW, Accessory molecules involved in antigen-mediated cytolysis and lymphokine production by cytotoxic T lymphocyte subsets. I. Identification of functions for the T cell surface molecules Ly-6C and Thy-1, J Immunol 151 (1993) 2986–2999. [PubMed: 8104217]

[30]. Havran WL, Lancki DW, Moldwin RL, Dialynas DP, Fitch FW, Characterization of an anti-Ly-6 monoclonal antibody which defines and activates cytolytic T lymphocytes, J Immunol 140 (1988) 1034–1042. [PubMed: 3257767]

[31]. Walunas TL, Bruce DS, Dustin L, Loh DY, Bluestone JA, Ly-6C is a marker of memory CD8+ T cells, J Immunol 155 (1995) 1873–1883. [PubMed: 7543536]

[32]. Lee HW, Park SJ, Choi BK, Kim HH, Nam KO, Kwon BS, 4-1BB promotes the survival of CD8+ T lymphocytes by increasing expression of Bcl-xL and Bfl-1, J Immunol 169 (2002) 4882–4888. [PubMed: 12391199]

[33]. Lee J, Lee EN, Kim EY, Park HJ, Chang CY, Jung DY, Choi SY, Lee SK, Lee KW, Kwon GY, Joh JW, Kim SJ, Administration of agonistic anti-4-1BB monoclonal antibody leads to the amelioration of inflammatory bowel disease, Immunol Lett 101 (2005) 210–216. [PubMed: 16026855]

[34]. Maerten P, Geboes K, De Hertogh G, Shen C, Cadot P, Bullens DM, Van Assche G, Penninckx F, Rutgeerts P, Ceuppens JL, Functional expression of 4-1BB (CD137) in the inflammatory tissue in Crohn's disease, Clin Immunol 112 (2004) 239–246. [PubMed: 15308117]

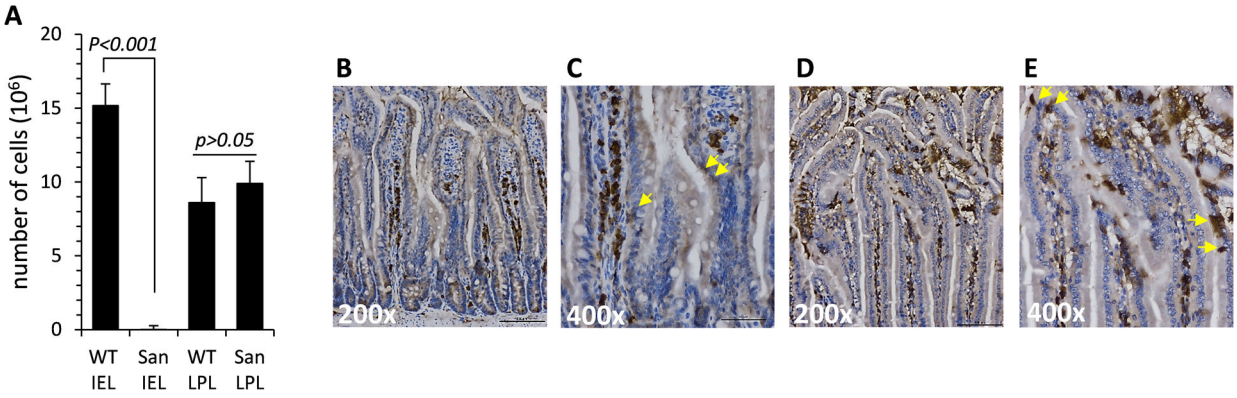

Fig. 1. T cell recoverability and cell dispersal in the sanroque intestinal mucosa.

(**A**) Numbers of IELs and LPLs recovered from nine WT and seven sanroque mice. (**B–C**) Representative small intestinal tissues stained for CD3 expression in WT mice. 200x and 400x original magnification, respectively. (**D–E**) Representative small intestinal tissues stained for CD3 expression in sanroque mice. 200x and 400x original magnification, respectively. Yellow arrows identify CD3+ cells 400x tissue sections in the epithelium and lamina propria of (**C**) WT and (**E**) sanroque mice.

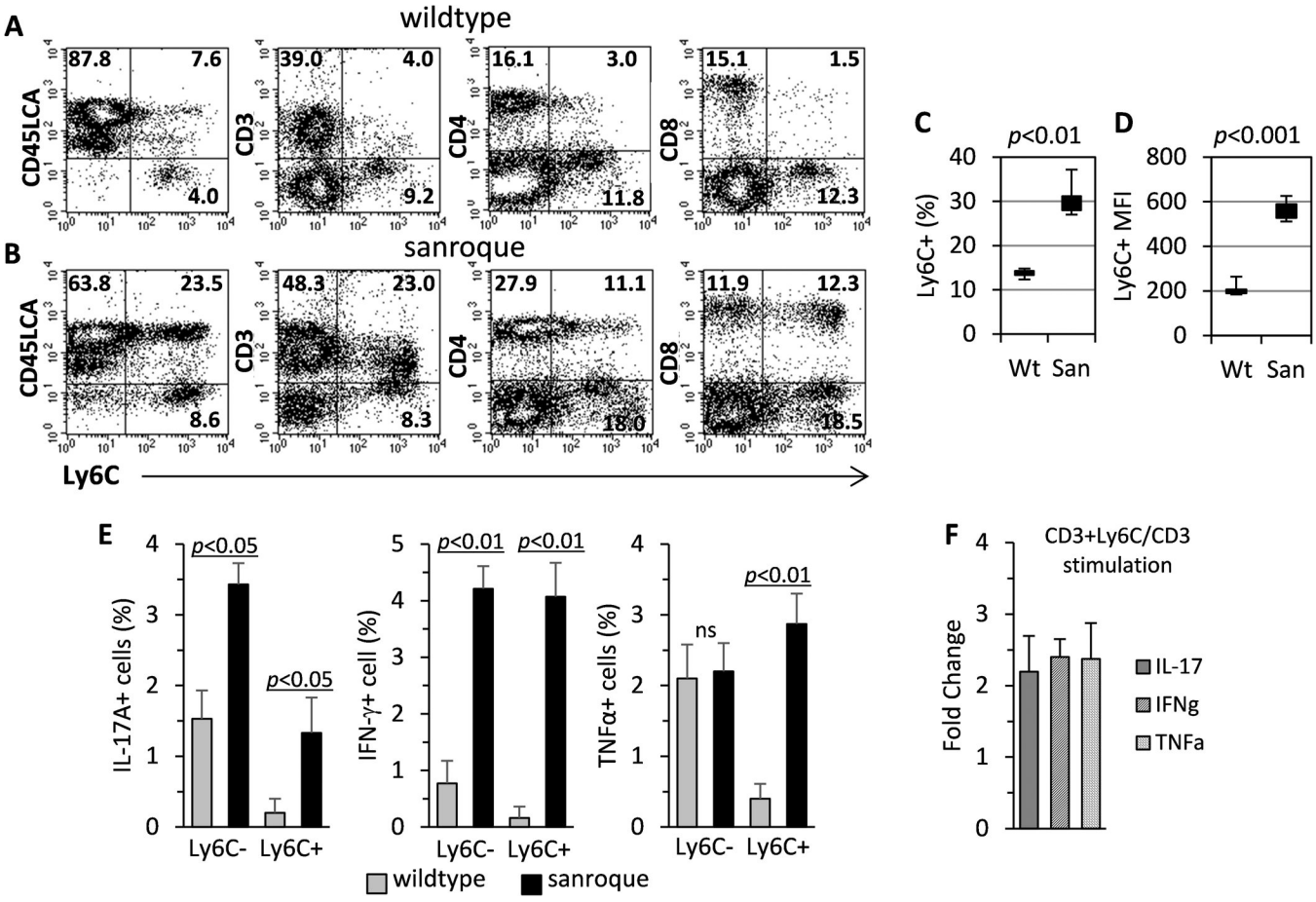

Fig. 2. Elevated Ly6C expression is linked to proinflammatory cytokine production.

(**A**) Expression of Ly6C on CD45LCA+, Cd3+, CD4+, and CD8+ LPLs from WT mice. (**B**) Expression of Ly6C on CD45LCA+, CD3+, CD4+, and CD8+ LPLs from sanroque mice. (**C**) Percent Ly6C+ cells on WT and sanroque LPLs; mean values ± sem of two mice each. (**D**) Mean fluorescence intensity of Ly6C expression on LPLs from WT and sanroque LPLs; mean values ± sem of two mice each. (**E**) Production of IL-17A, IFN-γ, and TNFα in Ly6C- and Ly6C+ LPLs from WT and sanroque mice; mean values ± sem of two mice each. (**F**) Fold change in proinflammatory cytokine production of LPLs from sanroque mice of CD3stimulation with Ly6C costimulation relative to CD3 stimulation alone; mean values ± sem of two mice.

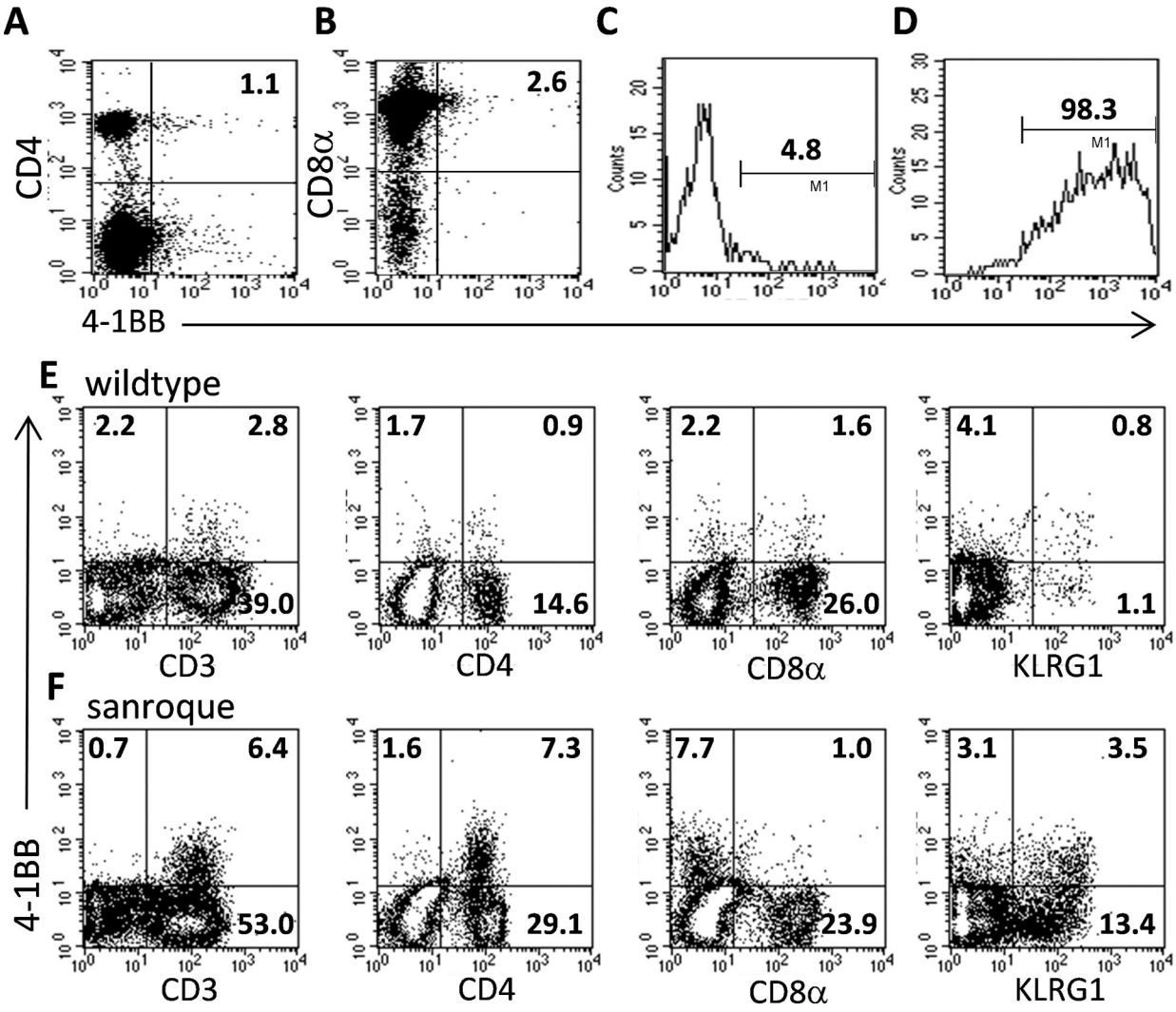

Fig. 3. 4-1BB expression on LPLs of WT and sanroque mice.

Resting (**A**) CD4+, (**B**) CD8+ T cells, and (**C**) Total T cells are devoid of 4-1BB expression, but (**D**) upregulate 4-1BB expression by 24 hr of CD3 stimulation. (**E**) Neither CD4+ nor CD8+ LPLs from WT mice express 4-1BB, whereas (**F**) some CD4+ LPLs from sanroque mice express 4-1BB. (**F**) 4-1BB expression on sanroque LPLs is linked to KLRG1 expression.

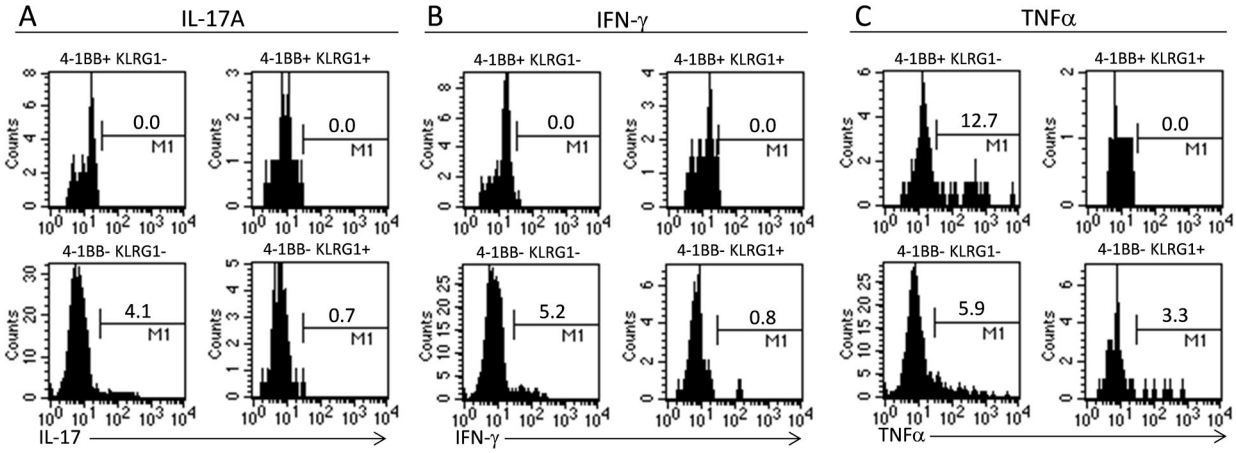

Fig. 4. Three color analyses of proinflammatory cytokine expression by sanroque LPLs based on 4-1BB and KLRG1 expression.

(**A**) IL-17A production by sanroque LPLs is not linked to 4-1BB or KLRG1 expression. (**B**) IFN-γ is produced by a subset of 4-1BB-, KLRG1- LPLs. (**C**) TNFα production is produced by three subsets of sanroque LPLs, 4-1BB+, KLRG1- cells, 4-1BB-, KLRG1- cells, and 4-1BB-, KLRG1+ cells.