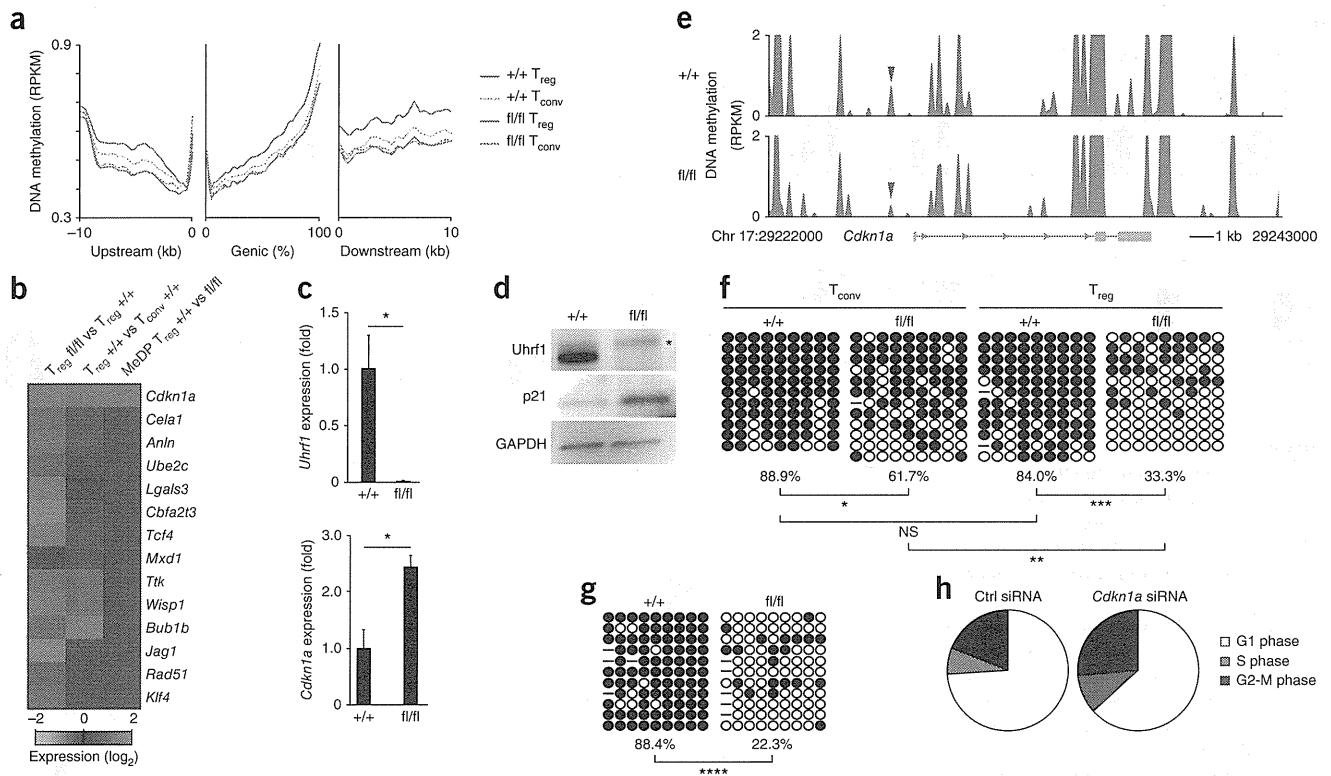

Figure 5 *Cdkn1a* is upregulated in Uhrf1-deficient T_{reg} cells because of hypomethylation of the *Cdkn1a* promoter region. (**a**) Genome-wide MeDP-seq analysis of DNA from *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre T_{conv} and T_{reg} cells, showing the average DNA methylation status (presented as 'reads' per kilobase of exon model per million mapped 'reads' (RPKM)) of upstream, genic and downstream regions. (**b**) Comparison of transcriptome analysis of *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre T_{reg} cells with the MeDP-seq analysis data to identify potential targets of Uhrf1 (right margin). (**c**) Quantitative PCR analysis of *Uhrf1* and *Cdkn1a* in *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre T_{reg} cells cultured *in vitro*; results are presented relative to those of *Uhrf1*$^{+/+}$*Cd4*-Cre cells, set as 1. $*P < 0.01$ (Student's *t*-test). (**d**) Immunoblot analysis of Uhrf1 and p21 in *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre T_{reg} cells cultured *in vitro*; GAPDH (glyceraldehyde phosphate dehydrogenase) serves as a loading control. *, nonspecific signal. (**e**) MeDP-seq analysis of *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre $CD3\varepsilon^+CD4^+hCD2^+$ cells *ex vivo*: red downward arrowheads, distal promoter region of *Cdkn1a* (exon structure below: orange, coding sequences; tan, UTR; arrows, direction of transcription). (**f**,**g**) Bisulfite-sequencing analysis of the methylation of CpG islands on the distal promoter region of *Cdkn1a* in *Uhrf1*$^{+/+}$*Cd4*-Cre and *Uhrf1*$^{fl/fl}$*Cd4*-Cre T_{conv} and T_{reg} cells *ex vivo* (**f**) and T_{reg} cells cultured *in vitro* (**g**): filled circles, methylated; open circles, demethylated; –, undetermined (due to noise signals); below, methylated CpG/total CpG. $*P = 5.94 \times 10^{-5}$, $**P = 4.34 \times 10^{-13}$, $***P = 7.18 \times 10^{-14}$ and $****P = 3.12 \times 10^{-21}$ (hypergeometric distribution). (**h**) Cell-cycle assay of *Uhrf1*$^{+/+}$*Cd4*-Cre naive $CD4^+$ T cells differentiated into $Foxp3^+$ cells, then treated for 24 h with fluorescence-labeled *Cdkn1a*-specific siRNA (*Cdkn1a* siRNA) or nontargeting (control) siRNA (Ctrl siRNA). Data are representative of two (**a**,**b**,**e**–**g**) or three (**c**,**d**,**h**) independent experiments.

Given that the absence of Uhrf1 led to a considerable defect in the accumulation of colonic T_{reg} cells, we reasoned that Uhrf1 might be fundamental to the maintenance of intestinal immunological homeostasis. In support of that proposal, *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice spontaneously developed colitis characterized by thickening of the colonic wall, epithelial hyperplasia, loss of goblet cells and massive cellular infiltrates into the colonic mucosa and submucosa before 10 weeks of age (**Fig. 6a–d** and **Supplementary Fig. 8a**). Nearly all of the *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice eventually succumbed to death within 6 months due to the exacerbated colitis (data not shown). In contrast, there were no inflammatory symptoms in the other peripheral tissues examined, including liver, kidney, lung, skin, pancreas, stomach, salivary gland and small intestine (**Supplementary Fig. 9**), consistent with the observation that the Uhrf1-regulated population expansion of T_{reg} cells occurred principally in the local colonic mucosa (**Figs. 1–4** and **Supplementary Figs. 1** and **2**). Commensal bacteria were the causative agent of this chronic inflammatory response, because *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice raised under GF conditions did not display any inflammation (**Supplementary Fig. 8b–d**).

We subsequently examined the immunological phenotype of the spontaneous colitis. The expression of genes encoding proinflammatory cytokines was upregulated considerably in *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice relative to their expression in *Uhrf1*$^{+/+}$*Cd4*-Cre mice (**Fig. 6e**). In keeping with that, the frequency of T_{eff} cells expressing the proinflammatory cytokines interferon-γ, IL-17A and tumor-necrosis factor in the cLP was much greater in *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice than in *Uhrf1*$^{+/+}$*Cd4*-Cre mice (**Fig. 6f,g**). Conversely, IL-10-expressing T_{reg} cells were nearly absent from colitic *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice (**Fig. 6f,g**). This was also the case even in younger mice before the development of frank colitis (**Fig. 4j**). These results suggested that activation of responses of the T_H1 and T_H17 subsets of helper T cells due to compromised T_{reg} cell function mediated the development of colitis in *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice.

To investigate the possibility that excessive activation of T_{eff} cells due to the loss of Uhrf1 might cause the development of colitis, we assessed the *in vivo* function of T_{eff} cells independently of the effect of T_{reg} cell dysfunction through the use of a mixed–bone marrow chimera system. We transferred $CD45.1^+$ wild-type and Uhrf1-deficient

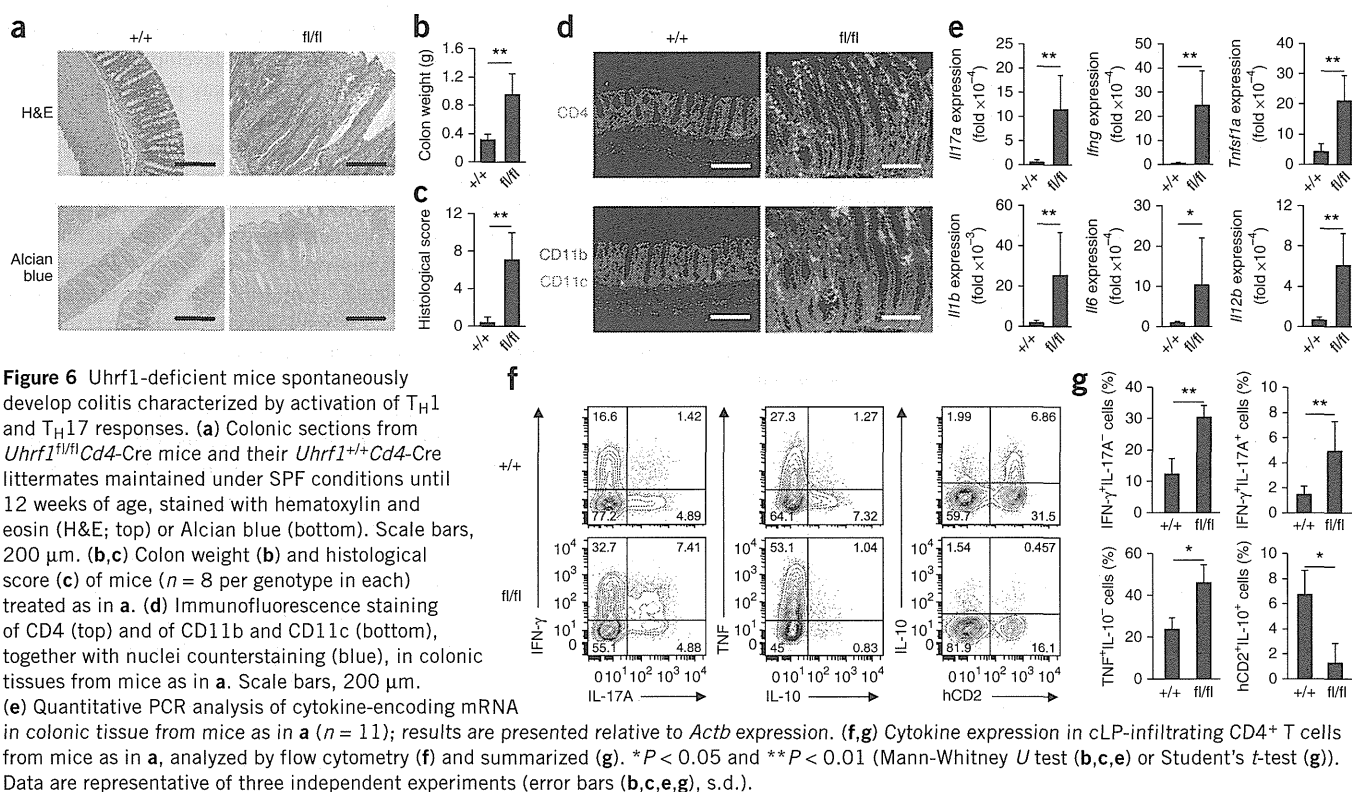

Figure 6 Uhrf1-deficient mice spontaneously develop colitis characterized by activation of T_H1 and T_H17 responses. (**a**) Colonic sections from *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice and their *Uhrf1*$^{+/+}$*Cd4*-Cre littermates maintained under SPF conditions until 12 weeks of age, stained with hematoxylin and eosin (H&E; top) or Alcian blue (bottom). Scale bars, 200 μm. (**b**,**c**) Colon weight (**b**) and histological score (**c**) of mice (*n* = 8 per genotype in each) treated as in **a**. (**d**) Immunofluorescence staining of CD4 (top) and of CD11b and CD11c (bottom), together with nuclei counterstaining (blue), in colonic tissues from mice as in **a**. Scale bars, 200 μm. (**e**) Quantitative PCR analysis of cytokine-encoding mRNA in colonic tissue from mice as in **a** (*n* = 11); results are presented relative to *Actb* expression. (**f**,**g**) Cytokine expression in cLP-infiltrating CD4⁺ T cells from mice as in **a**, analyzed by flow cytometry (**f**) and summarized (**g**). $*P < 0.05$ and $**P < 0.01$ (Mann-Whitney *U* test (**b**,**c**,**e**) or Student's *t*-test (**g**)). Data are representative of three independent experiments (error bars (**b**,**c**,**e**,**g**), s.d.).

bone marrow cells together into irradiated recipient mice with congenital deficiency in mature B cells and T cells (deficient in recombination-activating gene 1). We confirmed that these chimeras did not show any signs of inflammation in the colon (data not shown). Under these non-inflammatory conditions, the frequency of T_{eff} cells expressing interferon-γ and IL-17A in the cLP was similar for Uhrf1-deficient and Uhrf1-sufficient populations (**Supplementary Fig. 3d**). In addition, Uhrf1 deficiency did not influence the *in vitro* differentiation or function of the T_{eff} cells (**Supplementary Fig. 10a,b**). Examination of methylated DNA by MeDP-seq analysis also confirmed that the methylation status of genes encoding proinflammatory cytokines, as well as those encoding key transcription factors for T_H1 or T_H17 differentiation, was normal in the absence of Uhrf1 (**Supplementary Fig. 10c–f**). These results excluded the possibility that deficiency of Uhrf1 influenced the function of colonic T_{eff} cells.

We finally investigated whether the defect in T_{reg} cell proliferation was responsible for the development of colitis. To address this issue, we gave young (4- to 5-week-old) *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice wild-type T_{reg} cells (CD3⁺CD4⁺CD45.1⁺hCD2⁺ cells) from congenic *Foxp3*^{hCD2} reporter mice. Adoptive transfer of the wild-type T_{reg} cells prevented the development of colitis (**Fig. 7a**), concomitant with suppression of the T_{eff} cell response (**Fig. 7b**). Collectively, these data illustrated that the aberrant activation of T_{eff} cells caused by Uhrf1 deficiency resulted from the breakdown of the colonic immunoregulatory system. From this model, we concluded that the proliferative response of T_{reg} cells mediated by Uhrf1 was a prerequisite for their functional maturation in colonic mucosa.

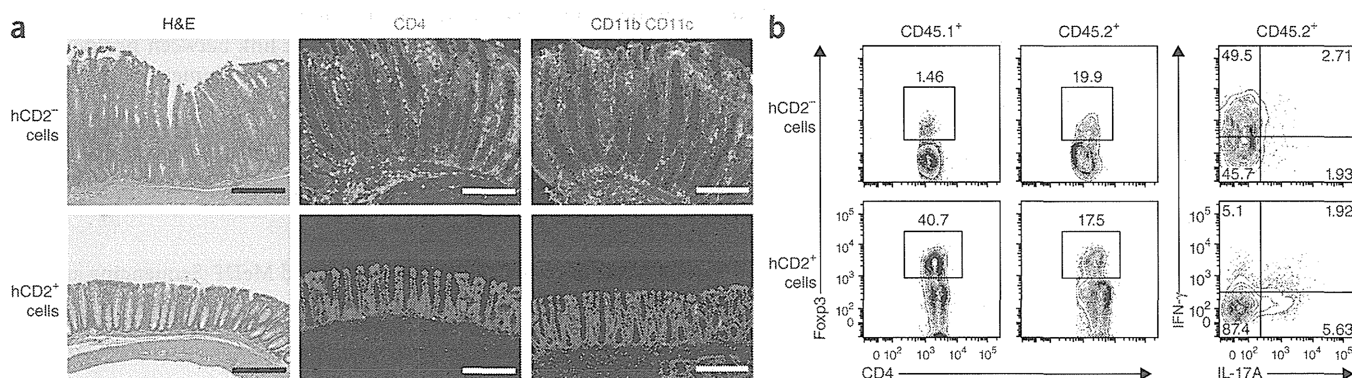

Figure 7 A defect in the proliferation of colonic T_{reg} cells is responsible for the development of spontaneous colitis in *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice. (**a**) Histology and immunofluorescence staining of colonic tissues from *Uhrf1*$^{fl/fl}$*Cd4*-Cre mice (6 weeks of age) at 6 weeks after transfer of hCD2⁺ or hCD2⁻ cells from CD45.1⁺ congenic *Foxp3*^{hCD2} mice. Scale bars, 200 μm. (**b**) Flow cytometry of CD4⁺ T cells from cLP of the mice in **a**. Numbers adjacent to outlined areas (left and middle) indicate percent Foxp3⁺CD4⁺ cells. Data are representative of two independent experiments (with a total of four mice per group) with similar results.

DISCUSSION

Multiple lines of evidence support the concept that dysregulation of the intestinal immune response to commensal microbes is a predisposing factor for inflammatory bowel disease[5,29–31]. Such chronic inflammatory responses compromise the homeostasis of the intestinal ecosystem and often result in dysbiosis[32,33]. Therefore, commensal microbes may have undergone adaptation to curtail host immune responses over the course of coevolution[1–3]. We have now demonstrated that bacterial colonization induced an early IL-2 response in the colonic mucosa that in turn led to the accumulation of T_{reg} cells, at least in part through vigorous proliferation, that overwhelmed the activation of T_{eff} cells. The local proliferative activity of colonic T_{reg} cells was maximal before weaning and gradually decreased with age. This suggested that vigorous proliferation of colonic T_{reg} cells was induced early after birth in parallel with the establishment of the commensal microflora. This model was congruent with the observation that the expression of *Uhrf1* was much higher in colonic T_{reg} cells from 2-week-old infant mice than in those from adult mice. Similarly, *Uhrf1* expression by colonic T_{reg} cells was upregulated after the inoculation of GF mice with intestinal microflora. In contrast, *Uhrf1* expression by splenic T_{reg} cells of the same mice remained unchanged before and after the inoculation, consistent with the minimal proliferative response in the spleen. Thus, the expression of *Uhrf1* was positively correlated with the proliferative activity of T_{reg} cells, and Uhrf1 deficiency had a substantial effect on the local population expansion of T_{reg} cells in response to bacterial colonization. The data as a whole supported our conclusion that local proliferation of T_{reg} cells was the main downstream consequence of Uhrf1 expression. Notably, the ablation of Uhrf1 had a substantial effect on the suppressive function of T_{reg} cells. We propose that this defect was due to the compromised proliferative response of Uhrf1-deficient T_{reg} cells, because proliferating T_{reg} cells had higher expression of functional molecules than did cells in the nonproliferative compartment. Therefore, colonic tissue acts as a privileged site in conferring functional maturity on T_{reg} cells. Given that Uhrf1-deficient mice spontaneously developed colitis, this immunoregulatory mechanism ensured by Uhrf1-dependent proliferation of T_{reg} cells was essential for the establishment of a symbiotic host-microbe relationship without inflammation.

Our data identified Uhrf1 as an IL-2-responsive molecule. In the intestine, both T cells and dendritic cells can produce IL-2 (refs. 34,35). We also confirmed that IL-2 was produced by both T cell populations and non-T cell populations, among which $CD4^+$ T cells mainly contributed to IL-2 production after colonization by commensals (data not shown). We found that colonization with the '17-mix' strains of Clostridia from human feces[23] drove T_{conv} cells to produce IL-2, which in turn upregulated *Uhrf1* in T_{reg} cells; this resulted in their active proliferation. In our *ex vivo* experiments, T_{conv} cells from mice colonized with 17-mix produced IL-2 only in the presence of autoclaved 17-mix (data not shown), which indicated that some of the T_{conv} cells in the mice colonized with 17-mix produced IL-2 in an antigen-specific manner. These observations raise the possibility that stimulation of T cells through the T cell antigen receptor with bacterial antigens may initiate activation of the IL-2–Uhrf1 pathway.

Like Uhrf1-deficient mice, mice lacking either IL-2 or one of its receptors (IL-2Rα or IL-2Rβ) spontaneously develop chronic colitis due to an excessive response to commensal bacteria[36,37]. Moreover, these mice develop lethal lymphoid hyperplasia and autoimmune disorders characterized by hemolytic anemia[38,39]. We did not observe such systemic autoimmune disorders in Uhrf1-deficient mice. Therefore, among the many biological functions of IL-2, the role of the IL-2–Uhrf1 pathway is itself confined to the maintenance of gut immunological homeostasis. Given that genetic polymorphisms in *IL2* and *IL2RA* are closely associated with the development of human inflammatory bowel disease[40], our findings may provide molecular insight into the pathogenesis of this disease.

We identified *Cdkn1a* (which encodes p21) as a target of Uhrf1 and showed the importance of the Uhrf1-p21 axis in the proliferation of T_{reg} cells. p21 has a vital role in controlling the proliferation, differentiation and tumorigenesis of many cell types[41]. The mechanisms for the regulation of *Cdkn1a* transcription are not yet fully elucidated, although it seems to be regulated via multiple pathways that may be different in various cell types. A possible link between Uhrf1 and p21 has been reported in embryonic stem cells and HeLa human cervical cancer cells[42]. The authors of that study[42] speculate that Uhrf1 recruits the histone lysine methyltransferase G9a to the *Cdkn1a* promoter to achieve accumulation of the repressive histone modification H3K9me2. *Cdkn1a* has a proximal promoter and a distal promoter in which CpG islands and a CpG cluster, respectively, are present. In intestinal epithelial cells, the proximal promoter is almost completely unmethylated; however, the distal promoter is partially methylated, which is negatively correlated with *Cdkn1a* expression[43]. In agreement with that observation, deficiency in Uhrf1 led to aberrant expression of *Cdkn1a* due to hypomethylation of its distal promoter region in T_{reg} cells. It is well documented that signaling via transforming growth factor-β (TGF-β) transactivates *Cdkn1a* expression as a canonical pathway[44,45]. Given that TGF-β, which is abundant in the intestinal tissue, is essential for the induction and maintenance of T_{reg} cells, it is conceivable that intestinal T_{reg} cells may be under continuous pressure to upregulate *Cdkn1a*. In this context, Uhrf1-dependent methylation of CpG sites may function to prevent the unwanted *Cdkn1a* expression that leads to a disadvantage in the progression of T_{reg} cells through the cell cycle.

Taking all of the observations noted above into account, we propose a model for establishment of gut immunological homeostasis based on reciprocal interaction between T_{reg} cells and T_{eff} cells. First, colonizing bacteria should be initially recognized by antigen-presenting cells such as dendritic cells. Second, the antigen-loaded antigen-presenting cells elicit an early IL-2 response by stimulating T_{eff} cells through antigen presentation. Third, the early IL-2 reponse provides a cue for T_{reg} cells to proliferate and simultaneously upregulate *Uhrf1* expression. Fourth, Uhrf1 represses the cell cycle–dependent kinase inhibitor p21 via methylation of *Cdkn1a* (which encodes p21) to safeguard the continuing proliferation of T_{reg} cells. Fifth, the actively proliferating T_{reg} cells become functionally mature and in turn prevent excessive immune responses to the colonizing microbiota. In conclusion, our study has provided a new mechanistic link between proliferation-dependent maturation of T_{reg} cells and containment of the inflammatory response to commensal microbiota.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. GEO: microarray and MeDP-Sequencing analysis data, GSE56544.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

Y.O. and Y.F. did a large part of the experiments together with D.T., K.A., Y.F., M.T., T.I., T.O., Y.I.K. and K. Ha.; Y.O., Y.F., T.A.E. and J.S. analyzed the data; M.N., S.T. and S.H. provided materials; S.O. prepared GF mice; T.D., H.M., O.O., K. Ho., H.O. and H.K. provided experimental protocols and intellectual input into the study; T.D. and H.O. edited the manuscript; K. Ha. and H.K. conceived of the study; and K. Ha. designed the experiments, analyzed the data and wrote the manuscript (together with Y.O. and Y.F.).

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Atarashi, K. *et al.* Induction of colonic regulatory T cells by indigenous Clostridium species. *Science* **331**, 337–341 (2011).
2. Round, J.L. & Mazmanian, S.K. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc. Natl. Acad. Sci. USA* **107**, 12204–12209 (2010).
3. Geuking, M.B. *et al.* Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity* **34**, 794–806 (2011).
4. Round, J.L. *et al.* The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* **332**, 974–977 (2011).
5. Round, J.L. & Mazmanian, S.K. The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* **9**, 313–323 (2009).
6. Park, S.-G. *et al.* T regulatory cells maintain intestinal homeostasis by suppressing γδ T cells. *Immunity* **33**, 791–803 (2010).
7. Furusawa, Y. *et al.* Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* doi:10.1038/nature12721 (2013).
8. Smith, P.M. *et al.* The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* **341**, 569–573 (2013).
9. Arpaia, N. *et al.* Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* **504**, 451–455 (2013).
10. Singh, N. *et al.* Activation of gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity* **40**, 128–139 (2014).
11. Kim, S.V. *et al.* GPR15-mediated homing controls immune homeostasis in the large intestine mucosa. *Science* **340**, 1456–1459 (2013).
12. Berger, S.L., Kouzarides, T., Shiekhattar, R. & Shilatifard, A. An operational definition of epigenetics. *Genes Dev.* **23**, 781–783 (2009).
13. Zheng, Y. *et al.* Role of conserved non-coding DNA elements in the *Foxp3* gene in regulatory T-cell fate. *Nature* **463**, 808–812 (2010).
14. Miyao, T. *et al.* Plasticity of Foxp3+ T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells. *Immunity* **36**, 262–275 (2012).
15. Ohkura, N. *et al.* T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for Treg cell development. *Immunity* **37**, 785–799 (2012).
16. Bostick, M. *et al.* UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science* **317**, 1760–1764 (2007).
17. Sharif, J. *et al.* The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* **450**, 908–912 (2007).
18. Unoki, M., Nishidate, T. & Nakamura, Y. ICBP90, an E2F-1 target, recruits HDAC1 and binds to methyl-CpG through its SRA domain. *Oncogene* **23**, 7601–7610 (2004).
19. Nishiyama, A. *et al.* Uhrf1-dependent H3K23 ubiquitylation couples maintenance DNA methylation and replication. *Nature* **502**, 249–253 (2013).
20. Yadav, M. *et al.* Neuropilin-1 distinguishes natural and inducible regulatory T cells among regulatory T cell subsets *in vivo*. *J. Exp. Med.* **209**, 1713–1722 (2012).
21. Weiss, J.M. *et al.* Neuropilin 1 is expressed on thymus-derived natural regulatory T cells, but not mucosa-generated induced Foxp3+ T reg cells. *J. Exp. Med.* **209**, 1723–1742 (2012).
22. Fontenot, J.D., Rasmussen, J.P., Gavin, M.A. & Rudensky, A.Y. A function for interleukin 2 in Foxp3-expressing regulatory T cells. *Nat. Immunol.* **6**, 1142–1151 (2005).
23. Atarashi, K. *et al.* Treg induction by a rationally selected mixture of *Clostridia* strains from the human microbiota. *Nature* **500**, 232–236 (2013).
24. Itoh, K. & Mitsuoka, T. Characterization of clostridia isolated from faeces of limited flora mice and their effect on caecal size when associated with germ-free mice. *Lab. Anim.* **19**, 111–118 (1985).
25. Webster, K.E. *et al. In vivo* expansion of T reg cells with IL-2-mAb complexes: induction of resistance to EAE and long-term acceptance of islet allografts without immunosuppression. *J. Exp. Med.* **206**, 751–760 (2009).
26. Barthlott, T. *et al.* CD25+CD4+ T cells compete with naive CD4+ T cells for IL-2 and exploit it for the induction of IL-10 production. *Int. Immunol.* **17**, 279–288 (2005).
27. Deng, C., Zhang, P., Harper, J.W., Elledge, S.J. & Leder, P. Mice lacking p21CIP1/WAF1 undergo normal development, but are defective in G1 checkpoint control. *Cell* **82**, 675–684 (1995).
28. Powrie, F., Leach, M.W., Mauze, S., Caddle, L.B. & Coffman, R.L. Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. *Int. Immunol.* **5**, 1461–1471 (1993).
29. Sellon, R.K. *et al.* Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect. Immun.* **66**, 5224–5231 (1998).
30. Manichanh, C., Borruel, N., Casellas, F. & Guarner, F. The gut microbiota in IBD. *Nat Rev Gastroenterol Hepatol* **9**, 599–608 (2012).
31. Sartor, R.B. Microbial influences in inflammatory bowel diseases. *Gastroenterology* **134**, 577–594 (2008).
32. Clemente, J.C., Ursell, L.K., Parfrey, L.W. & Knight, R. The impact of the gut microbiota on human health: an integrative view. *Cell* **148**, 1258–1270 (2012).
33. Winter, S.E. *et al.* Host-derived nitrate boosts growth of *E. coli* in the inflamed gut. *Science* **339**, 708–711 (2013).
34. Granucci, F. *et al.* Inducible IL-2 production by dendritic cells revealed by global gene expression analysis. *Nat. Immunol.* **2**, 882–888 (2001).
35. Han, D. *et al.* Dendritic cell expression of the signaling molecule TRAF6 is critical for gut microbiota-dependent immune tolerance. *Immunity* **38**, 1211–1222 (2013).
36. Sadlack, B. *et al.* Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell* **75**, 253–261 (1993).
37. Ehrhardt, R.O., Lúdvíksson, B.R., Gray, B., Neurath, M. & Strober, W. Induction and prevention of colonic inflammation in IL-2-deficient mice. *J. Immunol.* **158**, 566–573 (1997).
38. Willerford, D.M. *et al.* Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. *Immunity* **3**, 521–530 (1995).
39. Suzuki, H. *et al.* Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor β. *Science* **268**, 1472–1476 (1995).
40. Jostins, L. *et al.* Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* **491**, 119–124 (2012).
41. Sherr, C.J. & Roberts, J.M. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev.* **13**, 1501–1512 (1999).
42. Kim, J.K., Estève, P.-O., Jacobsen, S.E. & Pradhan, S. UHRF1 binds G9a and participates in p21 transcriptional regulation in mammalian cells. *Nucleic Acids Res.* **37**, 493–505 (2009).
43. Yang, W., Bancroft, L. & Augenlicht, L.H. Methylation in the p21WAF1/cip1 promoter of Apc+/–, p21+/– mice and lack of response to sulindac. *Oncogene* **24**, 2104–2109 (2005).
44. Pardali, K. *et al.* Role of Smad proteins and transcription factor Sp1 in p21(Waf1/Cip1) regulation by transforming growth factor-β. *J. Biol. Chem.* **275**, 29244–29256 (2000).
45. Cordenonsi, M. *et al.* Links between tumor suppressors: p53 is required for TGF-β gene responses by cooperating with Smads. *Cell* **113**, 301–314 (2003).

ONLINE METHODS

Animal experiments. *Uhrf1*$^{fl/fl}$ mice (generated as in **Supplementary Fig. 2**) were backcrossed onto a C57BL/6 background. For the generation of mice with T cell-specific Uhrf1 deficiency, *Uhrf1*$^{fl/fl}$ mice were crossed with *Cd4*-Cre mice (The Jackson Laboratory) and then *Foxp3*^{hCD2} mice[14]. *Uhrf1*$^{fl/fl}$*Cd4*-Cre*Foxp3*^{hCD2} mice were housed under SPF conditions unless otherwise specified. IQI mice (CLEA Japan) were maintained in GF conditions in vinyl isolators in the animal facilities of the RIKEN Center for Integrative Medical Sciences and Graduate School of Medical Life Science, Yokohama City University. Feces from SPF C57BL/6 mice were suspended in PBS or were treated with 3% (vol/vol) chloroform in PBS to generate chloroform-resistant bacteria, and GF IQI and *Uhrf1*$^{fl/fl}$*Cd4*-Cre *Foxp3*^{hCD2} mice were inoculated with aliquots of those suspensions by intragastric intubation[1]. Mice treated with chloroform-resistant bacteria were maintained in the gnotobiotic vinyl isolator for 3–4 weeks. Gnotobiotic mice associated with the 17-strain mixture of Clostridia (17-mix) were generated as described[23].

For inhibition of the homing of extraintestinal T_{reg} cells to the gut[11], exGF mice were treated with a mixture (100 μg each per mouse) of mAb to integrin α4 (PS/2; Millipore) plus mAb to integrin β_7 (FIB504; Biolegend) or with control IgG (400533; Biolegend) on day 3 after bacterial colonization. The exGF mice were then subjected to an *in vivo* EdU-incorporation assay as described below.

Systemic population expansion of T_{reg} cells was induced as described[25]. SPF *Uhrf1*$^{fl/fl}$*Cd4*-Cre*Foxp3*^{hCD2} mice and their *Uhrf1*$^{+/+}$*Cd4*-Cre*Foxp3*^{hCD2} littermates were given intraperitoneal injection of complexes of IL-2 and mAb to IL-2 (JES6-1A12; R&D Systems) three times on days 0, 1 and 2, and proliferation of splenic T_{reg} cells was analyzed on day 5.

Protocols approved by Animal Studies Committees of RIKEN Yokohama Institute, The Institute of Medical Science, The University of Tokyo and Graduate School of Medical Life Science, Yokohama City University, were used for all animal experiments.

Preparation of lymphocytes. Lymphocytes from the cLP were prepared as described[46]. Colonic tissues were treated at 37 °C for 20 min with Hanks' balanced-salt solution (Wako Pure Chemical Industries) containing 1 mM dithiothreitol and 20 mM EDTA for removal of epithelial cells. The tissues were then minced and were dissociated for 30 min at 37 °C with collagenase solution containing 0.5 mg/ml collagenase (Wako Pure Chemical Industries) and 0.5 mg/ml DNase I (Roche Diagnostics), 2% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin and 12.5 mM HEPES, pH 7.2, in RPMI-1640 medium (Sigma-Aldrich) to obtain single-cell suspensions. After filtration, the single-cell suspensions were washed with 2% FCS in RPMI-1640 medium and were subjected to Percoll gradient separation. The spleen and mesenteric lymph nodes were mechanically disrupted into single-cell suspensions.

For quantitative PCR analysis, colonic mononuclear cells was subjected to cell sorting using FACSAriaII to isolate CD3ε$^+$CD4$^+$CD25$^+$FR4$^+$ or CD3ε$^+$CD4$^+$hCD2$^+$ T_{reg} cells, in IQI or Foxp3^{hCD2} reporter mice, respectively. Our preliminary experiments demonstrated that the CD3ε$^+$CD4$^+$CD25$^+$FR4$^+$ population almost exclusively consists of Foxp3$^+$ cells, consistent with a previous report[47].

Flow cytometry. The following mAbs were conjugated to biotin, fluorescein isothiocyanate, Alexa Fluor 488, phycoerythrin, peridinin chlorophyll protein–cyanine 5.5, phycoerythrin-indotricarbocyanine, allophycocyanin, Alexa Fluor 647, Alexa Fluor 700, allophycocyanin-Hilite7, eFluor 450, Pacific blue, Brilliant violet 421 or V500: anti–human CD2 (RPA-2.10), mAb to mouse CD25 (PC61), mAb to mouse CD44 (IM7), mAb to mouse CD45R/B220 (RA3-6B2), mAb to mouse CD62L (MEL-14), mAb to mouse Gr1 (RB6-8C5), mAb to mouse IL-2 (JES6-5H4), mAb to mouse interferon-γ (XMG1.2), mAb to mouse tumor-necrosis factor (MP6-XT22) and mAb to mouse Ter119 (TER-119; all from Biolegend); mAb to mouse CD3ε (145-2C11), mAb to mouse folate receptor 4 (eBio12A5), mAb to mouse Foxp3 (FJK-16s), mAb to mouse CTLA-4 (UC10-4B9) and mAb to mouse IL-10 (JES5-16E3; all from eBioscience); and mAb to mouse CD4 (GK1.5), mAb to mouse IL-17A (TC11-18H10.1) and mAb to mouse Ki67 (B56; all from BD Bioscience). Biotinylated polyclonal antibody to mouse Nrp1 (BAF566) was from R&D Systems.

For intracellular staining of cytokines, lymphocytes from the LP were cultured for 6 h in complete medium (RPMI-1640 medium containing 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 55 μM mercaptoethanol and 20 mM HEPES, pH 7.2) supplemented with 50 ng/ml PMA, 500 ng/ml ionomycin and GolgiPlug (BD Bioscience). The lymphocytes were then stained with mAb to CD3ε, mAb to CD4 and mAb to human CD2 (all identified above), followed by intracellular staining of interferon-γ, IL-17A, tumor-necrosis factor and IL-10 (antibodies identified above) with a Cytofix/Cytoperm kit (BD Bioscience). The stained samples were analyzed with a FACSCanto II or FACSAria II and with DIVA software (BD Biosciences) and FlowJo software, version 9.3.2 (Tomy Digital Biology).

***In vivo* EdU-incorporation assay.** For the detection of proliferating cells *in vivo*, GF and exGF mice received intraperitoneal injection of 3 mg EdU (5-ethynyl-2′-deoxyuridine) in 200 μl PBS, followed by administration of drinking water containing 0.8 mg/ml EdU for 2 d before the analysis. cLP cells that had incorporated EdU were visualized with a Click-it EdU Flow cytometry kit according to the manufacturer's instructions (Invitrogen).

Gene-expression profiling. Total RNA was extracted with TRIzol reagent (Life Technologies) according to a standard protocol and was subjected to microarray analysis with a GeneChip Mouse Genome 430 2.0 Array (Affymetrix). The data sets obtained were analyzed with GeneSpring GX 11 software (Agilent) and the Ingenuity pathway-analysis program (Ingenuity Systems).

Cell culture. CD3$^+$CD4$^+$CD44^{lo}CD62L^{hi} naive T cells were prepared from the spleen and lymph nodes by cell sorting as described above. Isolated naive CD4$^+$ T cells (5×10^5 cells per ml) were cultured for 3 d in complete RPMI-1640 medium supplemented with 5 ng/ml TGF-β and 10 ng/ml IL-2 (R&D Systems) and Dynabeads coated with mAb to CD3 and mAb to CD28 (Life Technologies) to induce differentiation into Foxp3$^+$ cells, then populations of differentiated cells were expanded up to an additional 4 d in the presence of 0.5 ng/ml TGF-β and 10 ng/ml IL-2. For cell-cycle analysis, induced T_{reg} cells were pulsed for 2 h with 10 μM EdU (Invitrogen). The cells were stained for EdU and 7-amino-actinomycin D with a Click-iT EdU flow cytometry kit before cell-cycle analysis with a FACSCanto II (BD) and FlowJo software, version 9.3.2 (Tomy Digital Biology).

***In vitro* suppression assays.** naive populations of CD3ε$^+$CD4$^+$hCD2$^+$ cells and CD3ε$^+$CD4$^+$CD62L^{hi}CD44^{lo} cells were purified as T_{reg} cells and responder cells, respectively, with the IMag Cell Separation System followed by cell sorting. For the preparation of antigen-presenting cells, splenocyte samples from C57BL/6J mice were depleted of Thy-1.2$^+$ cells and were irradiated with γ-irradiation (20 Gy). Responder cells labeled with carboxyfluorescein diacetate succinimidyl ester were cultured for 3 d together with T_{reg} cells at a ratio of 1:1 in the presence of antigen-presenting cells and mAb to CD3 (10 μg/ml; 145-2C11; eBioscience).

DNA-methylation analysis. Genomic DNA from CD3ε$^+$CD4$^+$hCD2$^+$ cells and CD3ε$^+$CD4$^+$hCD2$^-$ cells derived from mesenteric lymph nodes of male mice were extracted with an AllPrep DNA/RNA extraction kit (Qiagen), then were fragmented to approximately 200 base pairs by high-intensity focused ultrasound (Covaris) and were precipitated with histidine-tagged recombinant MBD1 ('methyl-CpG-binding-domain protein 1')[48]. After amplification by PCR, DNA fragments of the proper size were subjected to cluster generation and sequencing analysis with a HiSeq 1000 system (Illumina). Sequenced 'reads' were mapped to the mm9 assembly of the mouse genome (National Center for Biotechnology Information) with Bowtie software for the alignment of short DNA sequences. Peaks for each population were 'called' by model-based analysis of ChIP-seq data with a *P*-value threshold of less than 10^{-5}. The difference in methylation for a gene in one condition relative to its methylation in another condition was calculated with the normalized 'reads' mapped from 4 kilobases upstream to 4 kilobases downstream of its transcription start site. Transcription start sites were defined according to annotation on the Entrez database (National Center for Biotechnology Information).

Genomic bisulfite sequencing of the *Cdkn1a* promoter was done as described[17] with an EpiTect kit (Qiagen). The amplified fragments were cloned

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with a TOPO TA cloning kit (Invitrogen) and were subsequently sequenced with the BigDye Terminator Cycle Sequencing system (Applied Biosystems) and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The PCR primers were designed with MethPrimer software (Li laboratory, Department of Urology, University of California, San Francisco). The sequences of the primer sets were as follows: 5′-ATATGTTGGTTTTTGAAGAGGG-3′ and 5′-ATCCCAAAAAATCCCACTATATC-3′.

Quantitative PCR. Total RNA was isolated from colonic tissues with an RNeasy mini kit (Qiagen) and was subjected to reverse transcription with a ReverTra Ace kit according to the manufacturer's instructions (Toyobo). The cDNA samples were amplified with a Thermal Cycler Dice Real Time System (TAKARA BIO), SYBR premix Ex Taq (TAKARA BIO) and the primer sets specific for mouse genes (sequences in **Supplementary Table 1**).

ChIP–quantitative PCR analysis. The MAGnify ChIP system (Life Technologies) was used as described[7], with a few modifications, for ChIP assays. Splenic $CD4^+CD25^+$ T cells were cultured for 3 d with Dynabeads Mouse T-Activator CD3-CD28 (Life Technologies) in the presence of 10 ng/ml IL-2 and 5 ng/ml TGF-β. The cells were allowed to 'rest' for 6 h in RPMI-1640 medium (Sigma-Aldrich) containing 0.1% FBS and then were stimulated for 1.5 h with or without 100 ng/ml IL-2. The cells were fixed for 10 min at 37 °C (in a water bath) with 1% formaldehyde, and the reaction was quenched by the addition of 125 mM glycine. Crude nuclei were isolated in SDS lysis buffer and were sonicated with a Microson (Misonix) and then a focused ultrasonicator (Covaris S220; Covaris) for the generation of chromatin fragments approximately 100–700 base pairs in length. The acoustic parameters were optimized as follows: duty cycle, 5%; intensity, 140 W; cycle and burst: 200 and 5 min. After evaluation of sample quality with an Agilent 2100 Bioanalyzer (Agilent), the sheared chromatin samples were immunoprecipitated overnight at 4 °C under gentle rotation with magnetic Protein A/G beads immobilized with anti-STAT5 (9363; Cell Signaling) or rabbit IgG (MAGnify ChIP kit; Life Technologies). After extensive washing of samples, immunocomplexes were eluted for 30 min at 55 °C, then were treated for 1 h at 65 °C with proteinase K for reversal of crosslinking. After extraction of DNA, quantitative PCR analysis was done with the following primer set specific for the promoter region of *Uhrf1*: 5′-TCCCTTTCTCCTCTCCCAGG-3′ and 5′-CTGCCGGCTATGCTCACTTT-3′.

Transfection of siRNA. Cells were transfect with siRNA through the use of an Amaxa Nucleofector kit according to the manufacturer's protocol (Ronza) with minor modifications. For this, 4 μg of negative control siRNA or pooled siRNA targeting *Cdkn1a* conjugated to the fluorescent dye Hilyte 488 (Nippon Gene) was added to Nucleofector solution containing 1×10^6 cells, followed by electroporation (Program: X-001). The cell cycle of cells containing Hilyte 488 was analyzed with Hoechst 33342, a cell-permeable DNA-binding dye, 24 h after electroporation. Transfection efficiency was approximately 10–15%. The sequence of the *Cdkn1a*-specific siRNA was as follows: 5′-GUUGCGCCGUGAUUGCGAU-3′, 5′-CCAGCCUGACAGAUUUCUA-3′ and 5′-GAACGGUGGAACUUUGACU-3′.

Immunoblot analysis. For immunoblot analysis, whole-cell extracts were prepared in RIPA lysis buffer containing a 'cocktail' of protease inhibitors (Nacalai Tesque). Equal amounts of cell lysate were separated by 5–20% gradient SDS-PAGE (Biorad). After transfer, proteins on Immobilon-P membranes (Millipore) were probed with the following primary antibodies: mAb to p21 (SX118; BD Pharmingen), mAb to GAPDH (6C5; Santa Cruz) and polyclonal antibody to Uhrf1 (M-132; Santa Cruz), together with horseradish peroxidase–conjugated antibody to mouse IgG (7076; Cell Signaling Technology) and antibody to rabbit IgG (7074; Cell Signaling Technology). The specific binding of the antibodies was visualized by an enhanced chemiluminescence detection system (Nacalai Tesque) and a LAS-3000 luminescent image analyzer (Fuji Film).

Histology. Prefixed colonic tissue sections were deparaffinized and rehydrated and were stained with either hematoxylin and eosin or Alcian blue–nuclear fast red. Specimens were histologically examined for the assignment of scores for the degree of colitis based on the following criteria: inflammatory infiltrates, mucosal hyperplasia and loss of goblet cells.

Adoptive-transfer experiments. Experimental colitis was induced in mice with deficient in recombination-activating gene 1 ($Rag1^{-/-}$) by adoptive transfer of $CD4^+CD25^-CD45RB^{hi}$ T cells as described[28]. Splenocyte samples fromC57BL/6 mice were enriched for $CD4^+$ T cells with the IMag Cell Separation System. The resultant $CD4^+$ T cells were labeled with fluorescein isothiocyanate–conjugated antibody to mouse CD3ε (145-2C11; BD Biosciences) and phycoerythrin-conjugated antibody to mouse CD45RB (16A; BD Biosciences), and $CD3\varepsilon^+CD4^+CD45RB^{hi}$ cells were isolated by sorting with a FACSAria II (BD Biosciences). The $Rag1^{-/-}$ recipients were given 1×10^5 $CD4^+CD25^-CD45RB^{hi}$ T cells via the tail vein and were analyzed at 6 weeks after transfer. For the experiment in **Supplementary Figure 6**, $CD4^+CD25^+$ T cells from $Uhrf1^{fl/fl}$*Cd4*-Cre or $CD4^{Cre}Uhrf1^{+/+}$ mice (8×10^4 cells per mouse) were transferred to $Rag1^{-/-}$ recipients together with $CD4^+CD25^-CD45RB^{hi}$ T cells from $CD45.1^+$ C57BL/6 mice (1×10^5 cells per mouse).

In the experiment in **Figure 7**, $CD4^+hCD2^+$ or $hCD2^-$ T cells from the spleen and peripheral lymph nodes of $Foxp3^{hCD2}$ mice (2×10^6 cells per mouse) were injected intravenously into 4- to 5-week-old $Uhrf1^{fl/fl}$*Cd4*-Cre$Foxp3^{hCD2}$ mice. The development of colitis in recipient mice was analyzed at 12 weeks of age.

Generation of mixed–bone marrow chimeras. Bone marrow cells isolated from femora of wild-type ($CD45.1^+$; 1×10^6 cells per mouse) and $Uhrf1^{fl/fl}$ *Cd4*-Cre or $Uhrf1^{+/+}$*Cd4*-Cre mice ($CD45.2^+$; 1×10^7 cells per mouse) were injected intravenously into $Rag1^{-/-}$ mice treated with γ-irradiation (8 Gy) before the injection. Six weeks later, the cLP of the recipient mice was analyzed by flow cytometry.

Immunofluorescence staining. Immunofluorescence staining of cross-sections of colonic tissues was done as described[49].

Statistical analysis. Differences between two or more groups were analyzed by Student's *t*-test or one-way ANOVA followed by Tukey's test. When variances were not homogeneous, the data were analyzed by the nonparametrical Mann-Whitney *U*-test or the Kruskal-Wallis test followed by the Scheffé test.

46. Weigmann, B. *et al.* Isolation and subsequent analysis of murine lamina propria mononuclear cells from colonic tissue. *Nat. Protoc.* **2**, 2307–2311 (2007).
47. Yamaguchi, T. *et al.* Control of immune responses by antigen-specific regulatory T cells expressing the folate receptor. *Immunity* **27**, 145–159 (2007).
48. Morita, S. *et al.* Genome-wide analysis of DNA methylation and expression of microRNAs in breast cancer cells. *Int. J. Mol. Sci.* **13**, 8259–8272 (2012).
49. Obata, Y. *et al.* Epithelial cell-intrinsic Notch signaling plays an essential role in the maintenance of gut immune homeostasis. *J. Immunol.* **188**, 2427–2436 (2012).

V.　研究成果の刊行に関する一覧

研究成果の刊行に関する一覧表

執筆者氏名	論文題名	書籍全体の編集者名	書籍名	出版社名	出版地	ページ	出版年・月
藤井俊光、渡辺　守	巻頭 3. 炎症性腸疾患 生物学的製剤の課題と展望		消化器疾患　最新の治療 2015-2016	南江堂	東京		(in press)
岡田英理子、渡辺　守	下痢	井上智子、稲瀬直彦	緊急度・重症度からみた　症状別看護過程＋病態関連図　第 2 版	医学書院	東京	787-792	2014 年・11 月
岡田英理子、渡辺　守	便秘	井上智子、稲瀬直彦	緊急度・重症度からみた　症状別看護過程＋病態関連図　第 2 版	医学書院	東京	805-809	2014 年・11 月
渡辺　守	PART2 排泄のしくみと肝臓の機能　腸の病気	協力：橋本尚詞／坂井建雄／上西一弘／渡辺　守／朝比奈靖浩／巽英介／ロバート・ジャービック／中　好文／川北哲也／杉本久美子／三輪高喜	Newton　400 号記念　大特集	ニュートンプレス	東京	34(1):40-41	2014 年・11 月
和田祥城、大塚和朗、福田将義、松沢　優、竹中健人、荒木昭博、渡辺　守、林　武雅、三澤将史、豊嶋直也、石垣智之、工藤進英	大腸 LST の NBI 拡大観察	鶴田　修	胃と腸	医学書院	東京	49(12):1693-1704	2014 年・11 月
渡辺　守	特集／消化管の再生医療　序	吉川敏一	G.I.Research	先端医学社	東京	22(5):1-2	2014 年・10 月
和田祥城、大塚和朗、渡辺　守	8.治療法の選択 Case⑥.	田中信治	症例で身につける消化器内視鏡シリーズ　大腸腫瘍診断　改訂版	羊土社	東京	292-295	2014 年・10 月
和田祥城、工藤進英、渡辺　守	3.大腸④Dense, Irregular, Sparse pattern.	拡大内視鏡研究会	拡大内視鏡 極限に挑む	日本メディカルセンター	東京	231-233	2014 年・10 月
松沢　優、大島　茂、渡辺　守	特集　炎症・凝固反応とオートファジー　炎症性腸疾患とオートファジー	坂田洋一、鈴木宏治	Thrombosis Medicine	先端医学社	東京	4(3):246-252	2014 年・9 月
大塚和朗、荒木昭博、渡辺　守	【外科医が知っておくべき小腸疾患】 検査 カプセル内視鏡(解説/特集)	藤森俊二、他	臨床外科	医学書院	東京	69(9):1064-1068	2014 年・9 月
竹中健人、大塚和朗、渡辺　守	【トピックス】ワールドコングレスレポート　IBD のセッションを中心に The 2nd Annual Meeting of Asian Organization for Crohn's & Colitis (AOCC) -Blueprint for AOCC：　A Future Worth Creating	「IBD Research」編集委員会	IBD Research	先端医学社	東京	8(3):56-58	2014 年・9 月
渡辺　守	巻頭言	久松理一、渡辺憲治	現場のエキスパートが教える実践！ IBD 診療	医学出版	東京		2014 年・9 月
齊藤詠子、渡辺　守	抗 TNF-α 抗体療法抵抗例に対する戦略	鴻江俊治	臨牀と研究	大道學館出版部	福岡	91(8):58-61	2014 年・8 月
齊藤詠子、渡辺　守	【炎症性腸疾患診療の新たな展開】 Crohn 病　抗 TNF-α 抗体療法抵抗例に対する戦略(解説/特集)	鴻江俊治	臨牀と研究	大道學館出版部	福岡	91(8):1056-1059	2014 年・8 月

執筆者氏名	論文題名	書籍全体の編集者名	書籍名	出版社名	出版地	ページ	出版年・月
大塚和朗、渡辺大輔、小形典之、長堀正和、藤井俊光、齊藤詠子、和田祥城、荒木昭博、渡辺　守	【日常遭遇する大腸炎の鑑別-内視鏡を中心に】サルモネラ腸炎(解説/特集)	「INTESTINE」編集委員会	Intestine	日本メディカルセンター	東京	18(4):373-377	2014年・7月
齊藤詠子、渡辺　守	【Intestinal Failureへの挑戦】 クローン病におけるIntestinal Failureとその対策　内科の立場から(解説/特集).	鴻江俊治	消化と吸収	医事出版会	東京	36(3):318-321	2014年・7月
藤井俊光、渡辺　守	【内科疾患　最新の治療明日への指針】(第2章)消化器 Crohn病(解説/特集)		内科	南江堂	東京	113(6):1062-1065	2014年・6月
和田祥城、工藤進英、渡辺　守	シリーズ総編集 渡辺守(分担)	渡辺　守、田中信治	これで納得！画像で見ぬく消化器疾患 vol.2 大腸	医学出版	東京	2:142-144	2014年・4月
久松理一	外来で診る消化管疾患	監修：日比紀文、金井隆典	Medical Science Digest	北隆館	国内	51-53	2014年・8月

Ⅵ.　知的財産権・社会活動報告

知的財産権の出願・登録状況（予定を含む。）

種　類	受付（識別）番号	出願日
高後裕 特許取得	特許第 5526320 号	2014 年 4 月 25 日
高後裕 特許取得	特許第 5660508 号	2014 年 12 月 12 日

社会活動に関する一覧表

活動者名（所属施設）	会の名称および講演演題等	会場および新聞名等	活動年月日
渡辺　守 （東京医科歯科大学）	「症状を抑える治療から内視鏡での粘膜治癒を目指す治療へ」	週刊朝日「名医のセカンドオピニオン」	2015 年 1 月 16 日 掲載
渡辺　守 （東京医科歯科大学）	「今月のサイエンス」	慶應義塾医学部新聞	2014 年 11 月 20 日 掲載
渡辺　守 （東京医科歯科大学）	「潰瘍性大腸炎」	BS 日テレ「おはよう日曜診療所」	2014 年 10 月 5 日 放送
渡辺　守 （東京医科歯科大学）	「クローン病　MRI 検査有効　短時間、少ない負担」	読売新聞　朝刊	2014 年 7 月 13 日 掲載
渡辺　守 （東京医科歯科大学）	「炎症性腸疾患の診断と治療」（後編）	テレビ東京「話題の医学」	2014 年 4 月 13 日 放送
渡辺　守 （東京医科歯科大学）	「炎症性腸疾患の診断と治療」（前編）	テレビ東京「話題の医学」	2014 年 4 月 6 日 放送
金井　隆典 （慶應義塾大学医学部）	知られざる腸の世界 2	TBS ラジオ	2015 年 1 月 4 日
金井　隆典 （慶應義塾大学医学部）	第 51 回日本内科学会近畿支部生涯教育講演会 「炎症性腸疾患の診断と治療　Up-to-Date	メルパルク京都	2014 年 12 月 6 日
金井　隆典 （慶應義塾大学医学部）	続･腸寿のすすめ　健康人の便を移植するという試み	サンデー毎日	2014 年 9 月 28 日
鈴木　康夫 （東邦大学医療センター）	知って得する！(新)名医の最新治療【分子標的薬で､より長く症状を抑えることが可能　炎症性腸疾患（潰瘍性大腸炎、クローン病】	週刊朝日	2015 年 1 月 16 日
鈴木　康夫 （東邦大学医療センター）	【からだの質問箱】	読売新聞	2015 年 1 月 25 日
鈴木　康夫 （東邦大学医療センター）	第 104 回日本消化器病学会九州支部例会　第 98 回日本消化器内視鏡学会九州支部例会ランチョンセミナー【潰瘍性大腸炎に対する新規治療法の意義】	大分オアシスタワーホテル	2014 年 12 月 6 日
鈴木　康夫 （東邦大学医療センター）	第 141 回千葉市医師会　胃腸疾患研究会【潰瘍性大腸炎治療の新たな展開】	千葉市総合保健医療センター	2014 年 10 月 8 日
鈴木　康夫 （東邦大学医療センター）	鳥越俊太郎　医療の現場【潰瘍性大腸炎～患者急増の難病】	ＢＳ朝日	2014 年 8 月 22 日
鈴木　康夫 （東邦大学医療センター）	千葉市保健所難病講演会【クローン病　最新医療情報】	千葉市保健所	2014 年 7 月 26 日
鈴木　康夫 （東邦大学医療センター）	香川 IBD セミナー【潰瘍性大腸炎の新治療戦略～インフリキシマブの位置づけ～】	JR ホテルクレメント高松	2014 年 7 月 4 日
鈴木　康夫 （東邦大学医療センター）	第 112 回日本消化器内視鏡学会中国支部例会　ランチョンセミナー【IBD における生物学的製剤の有効性】	岡山コンベンションセンター	2014 年 6 月 29 日
鈴木　康夫 （東邦大学医療センター）	大腸肛門病学会教育セミナー　ランチョンセミナー【生物学的製剤を用いた炎症性腸疾患の新治療戦略】	品川プリンス	2014 年 5 月 25 日
安藤　朗 （滋賀医科大学消化器内科）	第 7 回あじまぁの会 腸内細菌と健康の関わり	ザ・ナハテラス	2015 年 1 月 30 日
安藤　朗 （滋賀医科大学消化器内科）	第 15 回綴喜・相楽臨床懇話会 腸内細菌と健康の関わり	ハイアットリージェンシー京都	2015 年 1 月 24 日
安藤　朗 （滋賀医科大学消化器内科）	IBD Young Academy 炎症性腸疾患の病態と腸内細菌の関わり	ホテルグランヴィア京都	2014 年 12 月 11 日

活動者名（所属施設）	会の名称および講演演題等	会場および新聞名等	活動年月日
安藤　朗 （滋賀医科大学消化器内科）	第102回日本消化器病学会中国支部例会 腸内細菌と健康の関わり	広島国際会議場	2014年11月29日
安藤　朗 （滋賀医科大学消化器内科）	第62回東三河内分泌疾患談話会 腸内細菌と健康の関わり	ホテルアソシア豊橋	2014年11月18日
安藤　朗 （滋賀医科大学消化器内科）	第1回久留米消化器 Seminar 炎症性腸疾患の病態と腸内細菌の関わり	ホテルマリターレ創世	2014年11月17日
安藤　朗 （滋賀医科大学消化器内科）	第22回沖縄大腸疾患研究会 炎症性腸疾患の病態と腸内細菌の関わり	ラグナガーデンホテル	2014年11月13日
安藤　朗 （滋賀医科大学消化器内科）	甲賀湖南医師会学術講演会 腸内細菌と健康の関わり	ホテルボストンプラザ草津	2014年10月30日
安藤　朗 （滋賀医科大学消化器内科）	第21回千葉エキスパートミーティング 炎症性腸疾患の病態における腸内細菌の役割	三井ガーデンホテル	2014年10月9日
安藤　朗 （滋賀医科大学消化器内科）	第36回日本臨床栄養学会総会 第35回日本臨床栄養協会総会 第12回大連合大会 腸内細菌と健康の関わり	JPタワーホール&カンファレンス	2014年10月5日
安藤　朗 （滋賀医科大学消化器内科）	湖南消化器勉強会 腸内細菌と健康の関わり	ライズヴィル都賀山	2014年9月11日
安藤　朗 （滋賀医科大学消化器内科）	第3回エビデンスに基づく統合医療研究会 腸内細菌と健康の関わり	リーガロイヤル NBC 中之島センタービル	2014年8月2日
安藤　朗 （滋賀医科大学消化器内科）	湖光会学術講演会 腸内細菌と健康の関わり	琵琶湖ホテル	2014年7月19日
安藤　朗 （滋賀医科大学消化器内科）	第33回大腸病態治療研究会 IBDにおける腸内細菌叢の変化とその意義	ホテルグランヴィア大阪	2014年6月26日
高後　裕 （旭川医科大学）	ヤクルト健康フォーラム	とかちプラザ	2014年11月15日
高後　裕 （旭川医科大学）	ヤクルト健康フォーラム	旭川トーヨーホテル	2014年10月18日

Ⅶ. 研 究 事 業 報 告

厚生労働科学研究費委託費難治性疾患等実用化研究事業
「独自の体外病態モデルによる難治性炎症性腸疾患の
革新的治療薬開発に関する研究」
平成26年度第1回総会プログラム

期日　平成26年 7月25日（金）13：00～14：00

場所　味の素㈱本社ビル（東京都中央区京橋 1-15-1）

研究代表者　　渡辺　守

（東京医科歯科大学消化器病態学）

事務局　東京医科歯科大学消化器病態学
担当　　土屋　輝一郎
TEL：03-5803-5877　　FAX：03-5803-0268
E-mail：ibd.gast@tmd.ac.jp

第１回総会について

１）演題発表について

⑴ スライドは、Power Pointで作成し、USBフラッシュメモリディスクまたはCD-ROMに保存したものをお持込み下さい。（Windows,Macintoshどちらも対応可能ですが、御自分のPC以外の機器でも試写してからお持ち下さい。）

⑵ 発表30分前までにB1会場スライド受付までご提出下さい。その際、試写（出力確認）も必ず行ってください。使用したメディアは、画面確認後その場でご返却いたします。

⑶ ご発表は４分、討論３分でお願い致します。今回プロジェクトごとの総括は設定しておりません。

⑷ 資料を配布される場合には、100部を７月２２日(火)17:00必着でIBD班事務局までお送りいただくか、当日12時30分までにB1会場までお持ち下さい。**当日、お持ちいただく場合、会場でのコピーは困難ですので、必ず配布出来る状態の資料を、100部ご準備してお持ち下さい。**

２）発表データについて

厚生労働省への報告の必要上、発表スライドファイルを当日複製させていただきますことをご了承下さい。不都合のある先生におかれましては、事前に事務局まで御連絡お願いします。

３）会場セキュリティについて

⑴ 一階玄関ホール総会受付にて芳名録へご署名後、セキュリティカードをお受け取りいただき、改札を通って地下一階会場へお進みください。

⑵ 館内はセキュリティ制ですのでセキュリティカードを必ず常時携行してください。退出される際にはカードをご返却ください。カードの紛失があると全館内のセキュリティに支障を来たしますので、くれぐれも紛失ならびにお持ち帰りにならないようご注意ください。

４）駐車場について

駐車スペースはご用意しておりませんので、公共の交通機関をご利用ください。

５）会場案内図 味の素㈱本社ビル 東京都中央区京橋1-15-1／TEL 03-5250-8111

① JR「東京駅」八重洲中央口（徒歩10分）
② 東京メトロ銀座線「京橋駅」６番出口（徒歩５分）
③ 都営浅草線「宝町駅」A-2出口（徒歩３分）
④ 東京メトロ日比谷線「八丁堀駅」北口（徒歩10分）

厚生労働科学研究費委託費難治性疾患等実用化研究事業
「独自の体外病態モデルによる難治性炎症性腸疾患の革新的治療薬開発に関する研究」班
平成26年度第1回総会プログラム

（敬称略）

平成２６年７月２５日（金）

開会（13：00）

Ⅰ．研究代表者挨拶　研究代表者：渡辺　守

Ⅱ．研究報告

プロジェクトの総合推進　（13：05 ～ 13：12）

担当：東京医科歯科大学医歯学総合研究科消化器病態学教授　渡辺　守

①腸管上皮幹細胞制御探索プロジェクト

a．疑似腸管モデルの構築に関わる技術開発　（13：12 ～ 13：19）

担当：東京医科歯科大学医歯学総合研究科消化器病態学教授　渡辺　守

腸管上皮初代培養細胞を用いた体外病態モデルの構築

○土屋輝一郎[1]、堀田伸勝[2]、福島啓太[3]、林　亮平[3]、日比谷秀爾[3]、水谷知裕[3]、大島　茂[3]、永石宇司[4]、岡本隆一[5]、中村哲也[1]、大塚和朗[6]、渡辺　守[3]（東京医科歯科大学消化管先端治療学[1]、腫瘍センター[2]、東京医科歯科大学消化器病態学[3]、東京医科歯科大学消化器内科[4]、東京医科歯科大学再生医療研究センター[5]、東京医科歯科大学光学医療診療部[6]）

b．腸管上皮幹細胞運命決定制御機構の解明　（13：19 ～ 13：26）

担当：京都大学医学研究科消化器内科学教授　千葉　勉

マウスモデルを用いた腸管上皮幹細胞運命決定機構の解析

千葉　勉[1]、○妹尾　浩[1]、山賀雄一[1]、松本善秀[1]、後藤規弘[1]、吉岡拓人[1]、丸野貴久[1]、中西祐貴[2]（京都大学・消化器内科学[1]、Sanford　- Burnham 医学研究所[2]）

②免疫制御探索プロジェクト

a．IBD 患者病勢特異的マーカーの探索　（13：26 ～ 13：33）

担当：北里大学北里研究所病院炎症性腸疾患先端治療センター　センター長　日比紀文

IBD粘膜固有層単核細胞分離技術を応用したエピゲノム、代謝研究とその人種差検討

○小林　拓[1]、竹内　修[1]、筋野智久[1]、中野　雅[1]、Shehzad Z. Sheikh[2]、Sally Coultard[3]、日比紀文[1]（北里大学北里研究所病院炎症性腸疾患先進治療センター[1]、University of North Carolina[2]、New Castle University[3]）

b．IBD モデルマウスにおける免疫病態解析　（13：33 ～ 13：40）

担当：大阪大学大学院医学系研究科免疫学教授　竹田　潔

虫垂リンパ組織による腸管恒常性維持機構の解析

○梅本英司、竹田　潔（大阪大学大学院医学系研究科免疫制御学）

③病態特異的腸内細菌探索プロジェクト

a．疾患特異的腸内細菌の探索 （13：40 ～13：47）

担当：慶應義塾大学医学部消化器内科教授　金井隆典

Clostridium butyricum の免疫制御機序の解明

◯金井隆典[1]、林　篤[1,2]、松岡克善[1]、竹下　梢[1]、三枝慶一郎[1]、新井万里[1]、清原裕貴[1]、水野慎大[1]、久松理一[1]（慶應義塾大学医学部消化器内科[1]、ミヤリサン製薬[2]）

b．病勢相関性腸内細菌の探索 （13：47 ～13：54）

担当：理化学研究所統合生命医科学研究センター　チームリーダー　本田賢也

病勢相関性腸内細菌の探索

◯本田賢也[1,2,5]、新　幸二[1,6]、田之上大[1]、永野勇治[1]、成島聖子[1]、須田　亙[3]、大島健志朗[3,5]、森田英利[4]、服部正平[3]（理化学研究所・統合生命医科学研究センター・消化管恒常性研究チーム[1]、慶應義塾大学医学部・微生物学免疫学教室[2]、東京大学大学院新領域創成科学研究科附属オーミクス情報センター[3]、麻布大学獣医学部・食品科学研究室[4]、JST・CREST[5]、JST・さきがけ[6]）

事務局連絡

閉会挨拶

（14：00 終了予定）

＜改訂版＞

厚生労働科学研究費委託費難治性疾患等実用化研究事業「独自の体外病態モデルによる難治性炎症性腸疾患の革新的治療薬開発に関する研究」平成26年度第2回総会プログラム

期日　平成27年 1月23日（金）10：30～12：30

場所　味の素㈱本社ビル（東京都中央区京橋 1-15-1）

研究代表者　　渡辺　守

（東京医科歯科大学消化器病態学）

事務局　東京医科歯科大学消化器病態学
担当　　土屋　輝一郎
TEL：03-5803-5877　　FAX：03-5803-0268
E-mail：ibd.gast@tmd.ac.jp

第２回総会について

1）演題発表について

⑴ スライドは、Power Point で作成し、USB フラッシュメモリディスクまたは CD-ROM に保存したものをお持込み下さい。（Windows, Macintosh どちらも対応可能ですが、御自分の PC 以外の機器でも試写してからお持ち下さい。）

⑵ 発表 30 分前までに B1 会場スライド受付までご提出下さい。その際、試写（出力確認）も必ず行ってください。使用したメディアは、画面確認後その場でご返却いたします。

⑶ **ご発表は５分、討論３分でお願い致します。**今回プロジェクトごとの総括は設定しておりません。

⑷ 資料を配布される場合には、**100 部を１月２０日㈫17:00 必着で** IBD 班事務局までお送りいただくか、当日９時 30 分までに B1 会場までお持ち下さい。**当日、お持ちいただく場合、会場でのコピーは困難ですので、必ず配布出来る状態の資料を、100 部ご準備してお持ち下さい。**

2）発表データについて

厚生労働省への報告の必要上、発表スライドファイルを当日複製させていただきますことをご了承下さい。不都合のある先生におかれましては、事前に事務局まで御連絡お願いします。

3）会場セキュリティについて

⑴ 一階玄関ホール総会受付にて芳名録へご署名後、セキュリティカードをお受け取りいただき、改札を通って地下一階会場へお進みください。

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4）駐車場について

駐車スペースはご用意しておりませんので、公共の交通機関をご利用ください。

5）会場案内図 味の素㈱本社ビル 東京都中央区京橋 1-15-1／TEL 03-5250-8111

① JR「東京駅」八重洲中央口（徒歩 10 分）
② 東京メトロ銀座線「京橋駅」6 番出口（徒歩 5 分）
③ 都営浅草線「宝町駅」A-2 出口（徒歩 3 分）
④ 東京メトロ日比谷線「八丁堀駅」北口（徒歩 10 分）

厚生労働科学研究費委託費難治性疾患等実用化研究事業
「独自の体外病態モデルによる難治性炎症性腸疾患の
革新的治療薬開発に関する研究」班
平成26年度第2回総会プログラム

(敬称略)

平成27年1月23日（金）

開会（10：30）

Ⅰ．研究代表者挨拶　研究代表者：渡辺　守

Ⅱ．研究報告

プロジェクトの総合推進　（10：35 ～ 10：43）

担当：東京医科歯科大学医歯学総合研究科消化器病態学教授　渡辺　守

①腸管上皮幹細胞制御探索プロジェクト

a．疑似腸管モデルの構築に関わる技術開発　（10：43 ～ 10：51）

担当：東京医科歯科大学医歯学総合研究科消化器病態学教授　渡辺　守

腸管上皮初代培養細胞を用いた体外病態モデルの構築

◯土屋輝一郎[1]、堀田伸勝[2]、福島啓太[3]、林　亮平[3]、日比谷秀爾[3]、水谷知裕[3]、大島　茂[3]、永石宇司[4]、岡本隆一[5]、中村哲也[1]、大塚和朗[6]、渡辺　守[3]（東京医科歯科大学消化管先端治療学[1]、腫瘍センター[2]、東京医科歯科大学消化器病態学[3]、東京医科歯科大学消化器内科[4]、東京医科歯科大学再生医療研究センター[5]、東京医科歯科大学光学医療診療部[6]）

b．腸管上皮幹細胞運命決定制御機構の解明　（10：51～ 11：07）

担当：京都大学医学研究科消化器内科学教授　千葉　勉

マウスと体外モデルを併用した腸管上皮幹細胞維持機構の解析

千葉　勉[1]、◯妹尾　浩[1]、山賀雄一[1]、松本善秀[1]（京都大学・消化器内科学[1]）

特殊な腸管上皮M細胞分化の分子メカニズム

◯金谷高史[1]、佐藤俊朗[2]、大野博司[1]（理化学研究所統合生命医科学研究センター[1]、慶應義塾大学医学部消化器内科[2]）

②免疫制御探索プロジェクト

a．IBD患者病勢特異的マーカーの探索　（11：07～ 11：23）

担当：北里大学北里研究所病院炎症性腸疾患先端治療センター　センター長　日比紀文

IBD粘膜固有層単核細胞分離技術を応用したエピゲノム、代謝研究とその人種差検討

◯小林　拓[1]、竹内　修[1]、黒沼　智[1]、中野　雅[1]、Shehzad Z. Sheikh[2]、Sally Coultard[3]、日比紀文[1]
（北里大学北里研究所病院炎症性腸疾患先進治療センター[1]、University of North Carolina[2]、Newcastle University[3]）

クローン病における酸化ストレスマーカーの検討

松本主之、山本一成、◯千葉俊美、中村昌太郎（岩手医科大学消化器内科消化管分野）

b．IBDモデルマウスにおける免疫病態解析　（11：23 ～ 11：47）

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大腸上皮特異的分子による腸管炎症制御機構

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DSS腸炎に対する尿酸の抗炎症効果の検討

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