

(Polysciences, Warrington, PA). The size of each spot was measured with Zeiss KS ELISPOT software (Oberkochen, Germany).

In vitro culture. CD11b⁺ IgA⁺ or CD11b⁻ IgA⁺ PCs (10⁴ cells per well) were purified from the iLP and cultured with 100 ng ml⁻¹ phorbol 12-myristate 13-acetate plus 300 ng ml⁻¹ ionomycin, or 10 µg ml⁻¹ lipopolysaccharide (all from Sigma-Aldrich), for 24 h.

For the bacteria uptake assay, fluorescent *Staphylococcus aureus* was opsonized in accordance with the manufacturer's protocol (Molecular Probes). Mononuclear cells isolated from the iLP (2 × 10⁵ cells) were incubated with 1 × 10⁵ opsonized bacteria for 90 min. After being washed, the cells were stained with antibodies for PE-IgA (mA-6E1, 0.5 µg ml⁻¹, eBioscience) and Pacific Blue CD11b, and the bacterial uptake by each population was examined by flow cytometry.

Microarray analysis. Microarray analysis was performed as we previously reported⁴⁷. Briefly, CD11b⁺ IgA⁺ and CD11b⁻ IgA⁺ cells were isolated from the iLP, and total RNA was extracted from them with an RNeasy kit (Qiagen, Dusseldorf, Germany). cRNA was hybridized with DNA probes on a GeneChip Mouse Genome 430 2.0 array (Affymetrix), washed and fluorescence-labelled in accordance with the standard amplification protocol developed by Affymetrix. The fluorescence intensity of each probe was taken to represent the raw expression level and was quantified with GeneChip Operating software (Affymetrix). Data obtained from two independent experiments were analysed with GeneSpring 7.3.1 software (Silicon Genetics). All microarray data have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus database (www.ncbi.nlm.nih.gov/geo/) under the accession no. GSE37225.

Statistics. Results were compared by a non-parametric Mann-Whitney's *U*-test and unpaired *t*-test (two tailed) (GraphPad Software, San Diego, CA).

References

- Macpherson, A. J., McCoy, K. D., Johansen, F. E. & Brandtzaeg, P. The immune geography of IgA induction and function. *Mucosal Immunol.* **1**, 11–22 (2008).
- Brandtzaeg, P. Function of mucosa-associated lymphoid tissue in antibody formation. *Immunol. Invest.* **39**, 303–355 (2010).
- Fagarasan, S. Intestinal IgA synthesis: a primitive form of adaptive immunity that regulates microbial communities in the gut. *Curr. Top Microbiol. Immunol.* **308**, 137–153 (2006).
- Macpherson, A. J. & Slack, E. The functional interactions of commensal bacteria with intestinal secretory IgA. *Curr. Opin. Gastroenterol.* **23**, 673–678 (2007).
- Slack, E. *et al.* Innate and adaptive immunity cooperate flexibly to maintain host-microbiota mutualism. *Science* **325**, 617–620 (2009).
- Woof, J. M. & Kerr, M. A. The function of immunoglobulin A in immunity. *J. Pathol.* **208**, 270–282 (2006).
- Fagarasan, S., Kawamoto, S., Kanagawa, O. & Suzuki, K. Adaptive immune regulation in the gut: T cell-dependent and T cell-independent IgA synthesis. *Annu. Rev. Immunol.* **28**, 243–273 (2010).
- Hayakawa, K., Hardy, R. R. & Herzenberg, L. A. Progenitors for Ly-1 B cells are distinct from progenitors for other B cells. *J. Exp. Med.* **161**, 1554–1568 (1985).
- Kantor, A. B. & Herzenberg, L. A. Origin of murine B cell lineages. *Annu. Rev. Immunol.* **11**, 501–538 (1993).
- Montecino-Rodriguez, E., Leathers, H. & Dorshkind, K. Identification of a B-1 B cell-specific progenitor. *Nat. Immunol.* **7**, 293–301 (2006).
- Kunisawa, J. *et al.* Sphingosine 1-phosphate regulates peritoneal B-cell trafficking for subsequent intestinal IgA production. *Blood* **109**, 3749–3756 (2007).
- Tsuiji, M. *et al.* Requirement for lymphoid tissue-inducer cells in isolated follicle formation and T cell-independent immunoglobulin A generation in the gut. *Immunity* **29**, 261–271 (2008).
- Gohda, M. *et al.* Sphingosine 1-phosphate regulates the egress of IgA plasmablasts from Peyer's patches for intestinal IgA responses. *J. Immunol.* **180**, 5335–5343 (2008).
- Mora, J. R. *et al.* Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science* **314**, 1157–1160 (2006).
- Cerutti, A., Chen, K. & Chorny, A. Immunoglobulin responses at the mucosal interface. *Annu. Rev. Immunol.* **29**, 273–293 (2011).
- Radbruch, A. *et al.* Competence and competition: the challenge of becoming a long-lived plasma cell. *Nat. Rev. Immunol.* **6**, 741–750 (2006).
- Weinstein, P. D. & Cebra, J. J. The preference for switching to IgA expression by Peyer's patch germinal center B cells is likely due to the intrinsic influence of their microenvironment. *J. Immunol.* **147**, 4126–4135 (1991).
- Hamada, H. *et al.* Identification of multiple isolated lymphoid follicles on the antimesenteric wall of the mouse small intestine. *J. Immunol.* **168**, 57–64 (2002).
- Talham, G. L., Jiang, H. Q., Bos, N. A. & Cebra, J. J. Segmented filamentous bacteria are potent stimuli of a physiologically normal state of the murine gut mucosal immune system. *Infect. Immun.* **67**, 1992–2000 (1999).
- Cebra, J. J., Jiang, H. Q., Boiko, N. V. & Tlaskalova-Hogenova, H. The role of mucosal microbiota in the development, maintenance, and pathologies of the mucosal immune system. In *Mucosal Immunology* 3rd edn (Mestecky, J. *et al.* eds) 335–368 (Academic Press, 2005).
- Tezuka, H. *et al.* Regulation of IgA production by naturally occurring TNF/iNOS-producing dendritic cells. *Nature* **448**, 929–933 (2007).
- Suzuki, K. *et al.* The sensing of environmental stimuli by follicular dendritic cells promotes immunoglobulin A generation in the gut. *Immunity* **33**, 71–83 (2010).
- Shapiro-Shelef, M. & Calame, K. Regulation of plasma-cell development. *Nat. Rev. Immunol.* **5**, 230–242 (2005).
- Patarowidjojo, M. *et al.* Leukocyte-cell adhesion: a molecular process fundamental in leukocyte physiology. *Immunol. Rev.* **114**, 67–108 (1990).
- Pabst, O. *et al.* Cutting edge: egress of newly generated plasma cells from peripheral lymph nodes depends on beta 2 integrin. *J. Immunol.* **174**, 7492–7495 (2005).
- Kunisawa, J. & Kiyono, H. A marvel of mucosal T cells and secretory antibodies for the creation of first lines of defense. *Cell Mol. Life Sci.* **62**, 1308–1321 (2005).
- Shroff, K. E., Meslin, K. & Cebra, J. J. Commensal enteric bacteria engender a self-limiting humoral mucosal immune response while permanently colonizing the gut. *Infect. Immun.* **63**, 3904–3913 (1995).
- Kunisawa, J. *et al.* Lack of antigen-specific immune responses in anti-IL-7 receptor alpha chain antibody-treated Peyer's patch-null mice following intestinal immunization with microencapsulated antigen. *Eur. J. Immunol.* **32**, 2347–2355 (2002).
- Schwartzberg, P. L., Mueller, K. L., Qi, H. & Cannons, J. L. SLAM receptors and SAP influence lymphocyte interactions, development and function. *Nat. Rev. Immunol.* **9**, 39–46 (2009).
- Maxwell, C. A., McCarthy, J. & Turley, E. Cell-surface and mitotic-spindle RHAMM: moonlighting or dual oncogenic functions? *J. Cell Sci.* **121**, 925–932 (2008).
- Cario, E. *et al.* Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial cell lines expressing Toll-like receptors. *J. Immunol.* **164**, 966–972 (2000).
- Griffin, D. O. & Rothstein, T. L. A small CD11b(+) human B1 cell subpopulation stimulates T cells and is expanded in lupus. *J. Exp. Med.* **208**, 2591–2598 (2011).
- Ivanov, I. I. *et al.* Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* **139**, 485–498 (2009).
- Hoyer, B. F. *et al.* Short-lived plasmablasts and long-lived plasma cells contribute to chronic humoral autoimmunity in NZB/W mice. *J. Exp. Med.* **199**, 1577–1584 (2004).
- Pasare, C. & Medzhitov, R. Control of B-cell responses by Toll-like receptors. *Nature* **438**, 364–368 (2005).
- Hou, B. *et al.* Selective utilization of Toll-like receptor and MyD88 signaling in B cells for enhancement of the antiviral germinal center response. *Immunity* **34**, 375–384 (2011).
- Rousset, F. *et al.* Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc. Natl. Acad. Sci. USA* **89**, 1890–1893 (1992).
- Defrance, T. *et al.* Interleukin 10 and transforming growth factor beta cooperate to induce anti-CD40-activated naive human B cells to secrete immunoglobulin A. *J. Exp. Med.* **175**, 671–682 (1992).
- Punnonen, J. *et al.* Soluble and membrane-bound forms of signaling lymphocytic activation molecule (SLAM) induce proliferation and Ig synthesis by activated human B lymphocytes. *J. Exp. Med.* **185**, 993–1004 (1997).
- Tokoyoda, K., Hauser, A. E., Nakayama, T. & Radbruch, A. Organization of immunological memory by bone marrow stroma. *Nat. Rev. Immunol.* **10**, 193–200 (2010).
- Fagarasan, S., Kinoshita, K., Muramatsu, M., Ikuta, K. & Honjo, T. In situ class switching and differentiation to IgA-producing cells in the gut lamina propria. *Nature* **413**, 639–643 (2001).
- Kang, H. S. *et al.* Signaling via LTbetaR on the lamina propria stromal cells of the gut is required for IgA production. *Nat. Immunol.* **3**, 576–582 (2002).
- Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S. & Medzhitov, R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* **118**, 229–241 (2004).
- Hiroi, T., Yanagita, M., Ohta, N., Sakaue, G. & Kiyono, H. IL-15 and IL-15 receptor selectively regulate differentiation of common mucosal immune system-independent B-1 cells for IgA responses. *J. Immunol.* **165**, 4329–4337 (2000).

45. Yan, M. *et al.* Identification of a receptor for BLyS demonstrates a crucial role in humoral immunity. *Nat. Immunol.* **1**, 37–41 (2000).
46. Nuchi, T. *et al.* Rice-based mucosal vaccine as a global strategy for cold-chain- and needle-free vaccination. *Proc. Natl Acad. Sci. USA* **104**, 10986–10991 (2007).
47. Terahara, K. *et al.* Comprehensive gene expression profiling of Peyer's patch M cells, villous M-like cells, and intestinal epithelial cells. *J. Immunol.* **180**, 7840–7846 (2008).

Acknowledgements

This work was supported by grants from the Program for Promotion of Basic and Applied Research for Innovations in Bio-oriented Industry (BRAIN to J.K.), the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grants-in-Aid for Young Scientists A (22689015 to J.K.), for Scientific Research on Innovative Areas (23116506 to J.K.), for Scientific Research S (23229004 to H.K.), for Scientific Research on Priority Area (19059003 to H.K.), for Challenging Exploratory Research (24659217 to J.K.) and for the Leading-edge Research Infrastructure Program (to J.K. and H.K.); and the Young Researcher Overseas Visits Program for Vitalizing Brain Circulation (Japan Society for the Promotion of Science) (to J.K., H.K., Y.K. and Y.G.); grants for JSPS Fellows (021-07124 to Y.K.); and grants from the Ministry of Health and Welfare of Japan (J.K. and H.K.), the New Energy and Industrial Technology Development Organization (to H.K.), the Global Center of Excellence Program of the Center of Education and Research for Advanced Genome-based Medicine (to H.K.), the Yakult Bio-Science Foundation (to J.K.) and DK085329 from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK, to I.I.).

Author contributions

J.K. planned the research and experiments, analysed data, wrote the paper and directed the research; M.G., E.H., I.I., M.H., Y.S., Y.G., C.P., I.I.I., R.S., L.A., T.W., S.S., Y.K. and S.S. conducted the immunological experiments; K.T. and S.A. provided key materials; and H.K. wrote the paper.

Additional information

Accession codes: Microarray data have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus database under series accession code GSE37225.

Supplementary Information accompanies this paper at <http://www.nature.com/naturecommunications>

Competing financial interests: The authors declare no competing financial interests.

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How to cite this article: Kunisawa, J. *et al.* Microbe-dependent CD11b⁺ IgA⁺ plasma cells mediate robust early-phase intestinal IgA responses in mice. *Nat. Commun.* **4**:1772 doi: 10.1038/ncomms2718 (2013).



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