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LETTERS

Epithelial-cell-intrinsic IKK-β expression regulates intestinal immune homeostasis

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Intestinal epithelial cells (IECs) provide a primary physical barrier against commensal and pathogenic microorganisms in the gastrointestinal (GI) tract, but the influence of IECs on the development and regulation of immunity to infection is unknown[1]. Here we show that IEC-intrinsic IκB kinase (IKK)-β-dependent gene expression is a critical regulator of responses of dendritic cells and $CD4^+$ T cells in the GI tract. Mice with an IEC-specific deletion of IKK-β show a reduced expression of the epithelial-cell-restricted cytokine thymic stromal lymphopoietin in the intestine and, after infection with the gut-dwelling parasite *Trichuris*, fail to develop a pathogen-specific $CD4^+$ T helper type 2 (T_H2) response and are unable to eradicate infection. Further, these animals show exacerbated production of dendritic-cell-derived interleukin-12/23p40 and tumour necrosis factor-α, increased levels of $CD4^+$ T-cell-derived interferon-γ and interleukin-17, and develop severe intestinal inflammation. Blockade of proinflammatory cytokines during *Trichuris* infection ablates the requirement for IKK-β in IECs to promote $CD4^+$ T_H2 cell-dependent immunity, identifying an essential function for IECs in tissue-specific conditioning of dendritic cells and limiting type 1 cytokine production in the GI tract. These results indicate that the balance of IKK-β-dependent gene expression in the intestinal epithelium is crucial in intestinal immune homeostasis by promoting mucosal immunity and limiting chronic inflammation.

The mucosal surface of the GI tract is a major site of pathogen entry, but the immune cells associated with the gut must remain hyporesponsive to food antigens and commensal bacteria or risk chronic inflammation. Despite this need for balance, the processes that orchestrate immune responses in the intestinal tract are still poorly defined. Previous studies *in vitro* have implicated IECs in the regulation of innate immunity and chronic inflammation, but a direct role for these cells in immunity to enteric pathogens has never been tested *in vivo*[2–5]. We employed the *Trichuris muris* infection model to investigate the role of IECs in regulating $CD4^+$ T-cell-mediated immunity and inflammation in the GI tract. *Trichuris* is a natural GI pathogen of mice and provides a model for human intestinal nematode infections that affect an estimated 1,000 million people worldwide[6]. *Trichuris*-specific $CD4^+$ T_H2 cells that produce the cytokines interleukin (IL)-4, IL-5 and IL-13 are required to mediate resistance to infection, whereas interferon (IFN)-γ-producing T_H1 cells are associated with chronic infection and, in some circumstances, severe intestinal inflammation[7–10].

Trichuris lives in close association with the intestinal epithelium[11] and induces nuclear factor (NF)-κB activation as measured by DNA-binding activity in IECs of the large intestine of genetically resistant mice (Fig. 1a). To determine whether IEC-intrinsic NF-κB activation

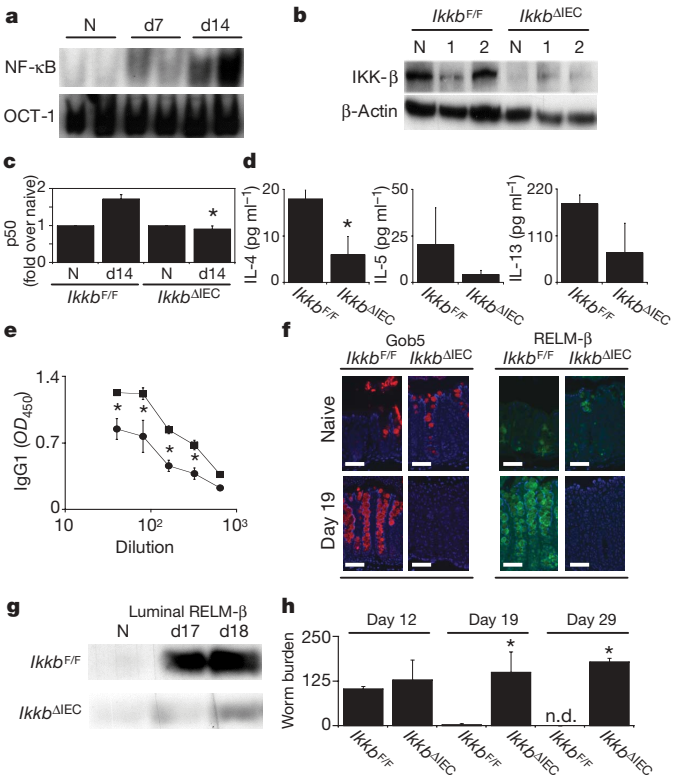

Figure 1 | IKK-β-dependent NF-κB activation in IECs is required for adaptive immunity to *Trichuris*. **a**, Infection-induced NF-κB activation in wild-type IECs from naive (N) and infected (days 7 and 14) animals. Assays were performed with either a consensus NF-κB-binding site probe or an octamer binding protein-1 OCT-1 probe as a loading control. **b**, IKK-β expression in IECs from naive (N) and infected (1 and 2) control $Ikkb^{F/F}$ or $Ikkb^{\Delta IEC}$ mice. **c**, NF-κB activation in IECs from naive (N) or infected control $Ikkb^{F/F}$ or $Ikkb^{\Delta IEC}$ mice determined by DNA capture ELISA. Data in **a**–**c** are representative of four to six individual animals from three independent experiments. **d**, *Trichuris*-specific cytokine responses after restimulation of mLN cells isolated from infected $Ikkb^{F/F}$ and $Ikkb^{\Delta IEC}$ mice. **e**, *Trichuris*-specific serum IgG1 levels (squares, $Ikkb^{F/F}$; circles, $Ikkb^{\Delta IEC}$). **f**, Gob5 and RELM-β expression in the caecum of naive or infected (day 19) $Ikkb^{F/F}$ or $Ikkb^{\Delta IEC}$ mice. Scale bar, 50 μm. **g**, Levels of secreted RELM-β isolated from faecal pellets of naive (N) and infected (days 17 and 18) $Ikkb^{F/F}$ or $Ikkb^{\Delta IEC}$ mice. **h**, Worm burdens at days 12, 19 and 29 after infection. n.d., not detected. Asterisk, $P < 0.05$. Error bars indicate s.e.m. ($n = 4$–6).

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influences immune responses in the GI tract, we examined the development of protective immunity in mice with an IEC-specific deletion of IKK-β (IKK-β/IKK-2). IKK-β is the catalytic subunit of the IKK complex responsible for NF-κB activation through the canonical pathway[12]. Mice in which the third exon of the *Ikkb* gene was flanked with *loxP* sites[13] ('floxed' *Ikkb*; $Ikkb^{F/F}$) were crossed with mice expressing the Cre recombinase from the IEC-specific *villin* promoter to generate mice in which IKK-β was specifically deleted in IECs ($Ikkb^{\Delta IEC}$ mice; Fig. 1b and refs 14–16). As a result, IECs isolated from $Ikkb^{\Delta IEC}$ mice fail to exhibit NF-κB activation after *Trichuris* infection (Fig. 1c). Analysis of the adaptive immune response revealed that, after infection, control $Ikkb^{F/F}$ mice developed polarized *Trichuris*-specific $CD4^+$ T_H2 responses characterized by increased production of IL-4, IL-5 and IL-13 after restimulation of draining mesenteric lymph node (mLN) cells with antigen (Fig. 1d). However, mLN cells isolated from infected $Ikkb^{\Delta IEC}$ mice showed decreased expression of protective $CD4^+$ T-cell-derived T_H2 cytokines (Fig. 1d and Supplementary Fig. 1a). Other parameters of type 2 immunity, including antibody isotype switching to IgG1 (Fig. 1e) and goblet cell responses, including hyperplasia (Supplementary Fig. 1b) and expression of Gob5 and RELM-β[17,18] (Fig. 1f, g), were impaired in the absence of IKK-β-dependent gene expression in IECs. Consistent with defective type 2 immune responses, $Ikkb^{\Delta IEC}$ mice were susceptible to infection, failing to clear parasites from the GI tract (Fig. 1h). IEC-intrinsic expression of IKK-β-dependent genes is therefore a crucial component in the development of $CD4^+$ T-cell-dependent protective immunity in the GI tract.

Results of studies *in vitro* have implicated cross-talk between IECs and intestinal dendritic cells (DCs) in influencing responses of DCs in the GI tract[3,19,20]. To determine whether the dysregulated $CD4^+$ T-cell response in infected $Ikkb^{\Delta IEC}$ mice was due to aberrant IEC–DC interactions, the composition and function of DC subsets in the gut-associated lymphoid tissue of $Ikkb^{F/F}$ or $Ikkb^{\Delta IEC}$ mice was examined after infection[21,22]. Similar frequencies of $CD11c^+CD11b^-CD8\alpha^-$ (double-negative, DN), $CD11c^+CD11b^-CD8\alpha^+$ ($CD8\alpha^+$) and $CD11c^+CD11b^+CD8\alpha^-$ ($CD11b^+$) DC subsets were observed in mLN of $Ikkb^{F/F}$ and $Ikkb^{\Delta IEC}$ mice before and after infection (Fig. 2a). Consistent with development of a protective type 2 response, no significant expression of the proinflammatory cytokines IL-12/23p40 or tumour necrosis factor (TNF)-α was observed by any DC subset isolated from infected $Ikkb^{F/F}$ mice (Fig. 2b, and data not shown). In contrast, there was an increased frequency of DN and $CD11b^+$ DCs expressing IL-12/23p40 and TNF-α in infected $Ikkb^{\Delta IEC}$ mice (Fig. 2b). The increased proinflammatory cytokine production could have been due to a loss of epithelial barrier function, because NF-κB controls the survival of IECs after exposure to sublethal doses of radiation or chemical inducers of colitis[14,15]. However, there was no evidence for defective barrier integrity in naive or infected $Ikkb^{\Delta IEC}$ mice (Supplementary Figs 2 and 3). These results therefore indicate that IEC-intrinsic IKK-β expression might have a crucial role in tissue-specific conditioning of DCs, specifically influencing the expression of proinflammatory cytokines independently of regulating barrier function.

To investigate which IKK-β-dependent genes within IECs could be responsible for dysregulated DC responses, we isolated RNA from the large intestine of naive $Ikkb^{F/F}$ or $Ikkb^{\Delta IEC}$ mice and analysed the expression of a panel of IEC-specific genes. Although we could not detect any significant differences in expression of the chemokines CCL6, CCL20 or CCL28 between naive and infected $Ikkb^{F/F}$ or $Ikkb^{\Delta IEC}$ mice (Supplementary Fig. 4), we observed significantly lower expression of the IEC-derived cytokine thymic stromal lymphopoietin (TSLP) in $Ikkb^{\Delta IEC}$ mice both before and after infection with *Trichuris* (Fig. 2c, d). The murine *Tslp* promoter contains two NF-κB-binding sites, which is consistent with a role for IKK-β in TSLP production[23]. To address whether *Tslp* messenger RNA expression in IECs is dependent on NF-κB activation, the murine

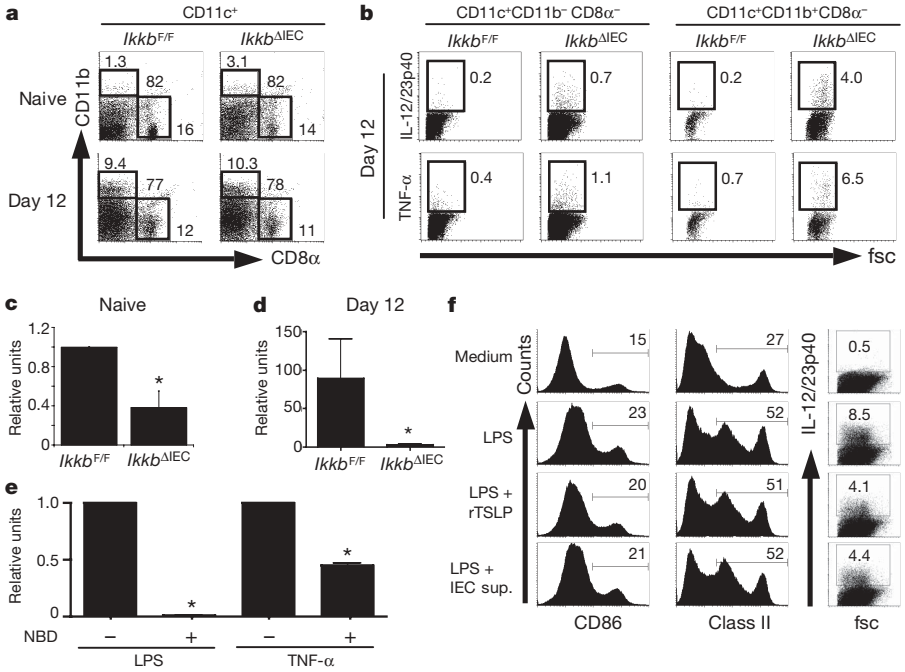

Figure 2 | Dysregulated dendritic cell responses in $Ikkb^{\Delta IEC}$ mice after infection with *Trichuris* are associated with defective TSLP expression. **a**, Composition of the dendritic cell compartment in the mLN of naive and infected (day 12) $Ikkb^{F/F}$ or $Ikkb^{\Delta IEC}$ mice. **b**, IL-12/IL-23p40 and TNF-α production by $CD11c^+CD11b^-CD8\alpha^-$ or $CD11c^+CD11b^+CD8\alpha^-$ cells from the draining mLN of infected (day 12) $Ikkb^{F/F}$ or $Ikkb^{\Delta IEC}$ mice. **c, d**, *Tslp* mRNA levels in the intestine of naive (**c**) and infected (**d**) $Ikkb^{F/F}$ or $Ikkb^{\Delta IEC}$ mice. Results in **c** represent fold induction over naive $Ikkb^{F/F}$ animals; results in **d** represent fold induction of infected $Ikkb^{F/F}$ or $Ikkb^{\Delta IEC}$ mice relative to respective naive $Ikkb^{F/F}$ or $Ikkb^{\Delta IEC}$ controls. **e**, *Tslp* mRNA levels in CMT-93 cells after treatment with IKK inhibitor peptide (NBD) or vehicle (dimethylsulphoxide) before stimulation with LPS or TNF-α. **f**, Bone-marrow-derived DCs were stimulated with LPS in the presence or absence of supernatants (sup.) from the IEC line CMT-93 or rTSLP and analysed for expression of surface markers and production of IL-12/23p40. Fsc, forward scatter. Asterisk, $P < 0.05$. Error bars indicate s.e.m. Data are representative of four independent experiments.

IEC line CMT-93 was stimulated with lipopolysaccharide (LPS) or TNF-α in the presence or absence of a peptide inhibitor of the IKK complex[24]. Stimulation of CMT-93 cells with LPS or TNF-α resulted in a fivefold and eightfold upregulation of *Tslp* mRNA by 24 h, respectively (data not shown). Treatment with the IKK inhibitor peptide NEMO-binding domain (NBD) resulted in a significant decrease in both LPS-induced and TNF-α-induced *Tslp* mRNA expression at 24 h after stimulation (Fig. 2e), indicating that IEC-intrinsic expression of TSLP might be regulated by IKK-β-dependent NF-κB activation.

Interactions between TSLP and its receptor (TSLPR) have been implicated in driving type 2 responses, either by providing a positive signal to DCs that governs their ability to promote T_H2 cell differentiation or by limiting the production of proinflammatory cytokines that promote T_H1 or T_H17 cell differentiation[25]. To determine whether IEC-derived products, including TSLP, could directly affect DC maturation and function, bone-marrow-derived DCs were activated with LPS in the presence or absence of either conditioned supernatants from a murine IEC line (CMT-93) or recombinant TSLP (rTSLP). The presence of IEC-conditioned supernatant or rTSLP had no effect on LPS-induced expression of CD86 or major histocompatibility complex class II (Fig. 2f, histograms). However, both IEC supernatant and rTSLP inhibited the production of LPS-induced IL-12/23p40 (Fig. 2f, dot plots). These data support the hypothesis that dysregulated IEC-intrinsic TSLP expression in $Ikkb^{\Delta IEC}$ mice contributes to heightened production of DC-derived proinflammatory cytokines.

Associated with decreased TSLP levels and enhanced expression of proinflammatory cytokines was a heightened expression of *Trichuris*-specific $CD4^+$ T-cell-derived IFN-γ in infected $Ikkb^{\Delta IEC}$ mice (Fig. 3a). Single-cell analysis revealed that the increased IFN-γ secretion in culture supernatants was due to both a heightened frequency of IFN-γ^+ $CD4^+$ T cells (Fig. 3a; $Ikkb^{F/F}$, 2.6%; $Ikkb^{\Delta IEC}$, 6.1%) and increased IFN-γ production per cell (Fig. 3a; mean fluorescence intensity: $Ikkb^{F/F}$, 314; $Ikkb^{\Delta IEC}$, 679). Increased production of IL-17, a cytokine implicated in the pathogenesis of several inflammatory conditions including chronic intestinal inflammation[26], was also detected in supernatants of antigen-restimulated mLN cells isolated from infected $Ikkb^{\Delta IEC}$ mice (Fig. 3b). Enhanced expression of proinflammatory cytokines in infected $Ikkb^{\Delta IEC}$ mice was accompanied by increased migration of $B220^+$ and $CD3^+$ lymphocytes into the intestine at day 19 after infection (Fig. 3c). Flow cytometric analysis of lamina propria cell populations showed increased frequencies of $CD4^+$ and $CD8^+$ T cells as well as $CD19^+$ B cells in infected $Ikkb^{\Delta IEC}$ mice compared with infected $Ikkb^{F/F}$ mice (Fig. 3d). The increased infiltration of lymphocytes into the lamina propria of infected $Ikkb^{\Delta IEC}$ mice at day 29 after infection was correlated with severe intestinal inflammation, disrupted crypt architecture, areas of focal ulceration and increased frequency of apoptotic IECs (Fig. 3e, and data not shown), which resulted in septicaemia (Fig. 3f). Thus, IEC IKK-β is critical for the delivery of signals that condition DCs in the gut to allow the development of $CD4^+$ T_H2 responses and prevent intestinal inflammation.

The ability of TSLP to limit the expression of proinflammatory cytokines by DCs, and its reduced expression in $Ikkb^{\Delta IEC}$ mice, led to the hypothesis that IEC-intrinsic IKK-β-dependent expression of TSLP is necessary to limit the production of cytokines that drive non-protective type 1 responses rather than to promote the expression of T_H2 cytokines directly. To test this, we used monoclonal antibodies to block the proinflammatory cytokines IL-12, IL-23 and IFN-γ in *Trichuris*-infected $Ikkb^{\Delta IEC}$ mice. This resulted in a decreased expression of antigen-specific IL-12-dependent IFN-γ and IL-23-dependent IL-17, and a concomitant increase in *Trichuris*-specific IL-13 after restimulation of cells isolated from mLN of treated $Ikkb^{\Delta IEC}$ mice (Fig. 4a, compare Ctrl with Tx). Consistent with increased type 2 and decreased type 1 cytokines, levels of *Trichuris*-specific IgG2a, an IFN-γ-dependent antibody isotype, were decreased in infected $Ikkb^{\Delta IEC}$ mice after treatment with monoclonal antibodies (Fig. 4b). In addition, blockade of IL-12, IL-23 and IFN-γ recovered goblet cell responses (Supplementary Fig. 5a), increased the expression of Gob5 and RELM-β (Fig. 4c and Supplementary Fig. 5b) and resulted in resistance to infection (Fig. 4d). The recovery of immunity in $Ikkb^{\Delta IEC}$ mice after blockade of proinflammatory cytokines shows that IEC-intrinsic IKK-β expression is not absolutely required for the promotion of type 2 immunity. Rather, IKK-β-dependent genes—including *Tslp*—seem to be a critical component of homeostatic processes that limit type 1 cytokine responses in the GI microenvironment. To test this hypothesis, DCs were isolated from the GI tract of mice deficient in TSLPR ($Tpte2^{-/-}$ mice). Consistent with a role for TSLP–TSLPR interactions in limiting DC proinflammatory cytokine production, there was an increased frequency (Fig. 4e) and absolute number (Fig. 4f) of DN and $CD11b^+$ DCs that expressed IL-12/23p40 in $Tpte2^{-/-}$ mice compared with controls. Similarly to results observed in *Trichuris*-infected $Ikkb^{\Delta IEC}$ mice, mLN cells isolated from infected $Tpte2^{-/-}$ mice produced significantly higher levels of antigen-specific IFN-γ (Fig. 4g). Other parameters of type 2 immunity, including *Trichuris*-specific serum IgG1 levels (Fig. 4h) and goblet cell hyperplasia (Fig. 4i), were also defective in $Tpte2^{-/-}$ mice. In addition,

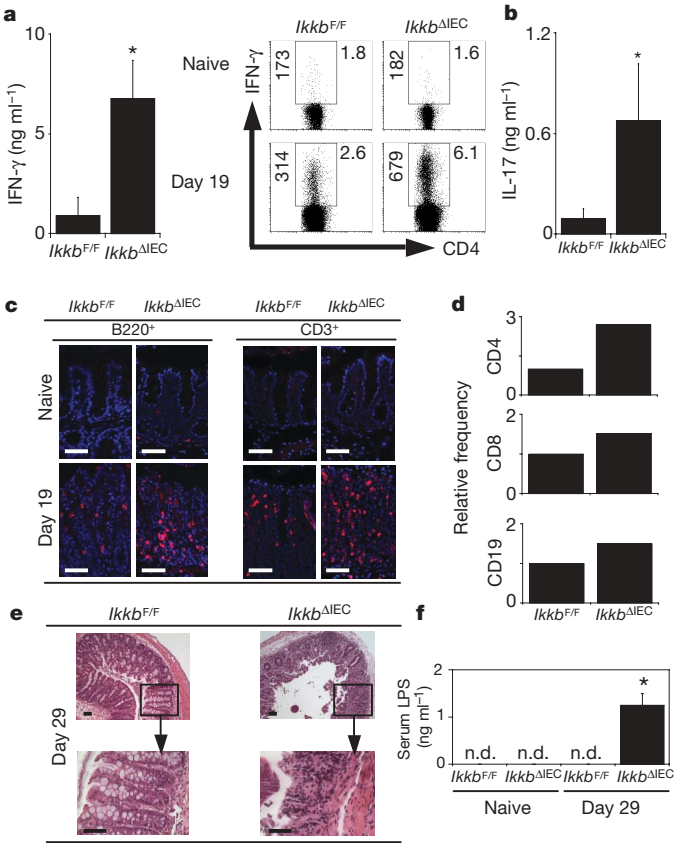

Figure 3 | $Ikkb^{\Delta IEC}$ mice show exacerbated pathogen-specific IFN-γ and IL-17 responses and severe intestinal inflammation after infection with *Trichuris*. **a**, **b**, *Trichuris*-specific IFN-γ (**a**) and IL-17 (**b**) responses after restimulation of cells isolated from mLN of naive and infected (day 19) $Ikkb^{F/F}$ or $Ikkb^{\Delta IEC}$ mice. **c**, **d**, Cell infiltration in the caecal mucosa of naive and infected $Ikkb^{F/F}$ or $Ikkb^{\Delta IEC}$ mice determined by immunofluorescent staining for B220 or CD3 (**c**) and by flow cytometry of isolated lamina propria cells (**d**). Scale bar, 50 μm. Data in **d** represent the relative increase in frequency of $CD4^+$, $CD8^+$ and $CD19^+$ cells in infected $Ikkb^{\Delta IEC}$ mice by comparison with infected $Ikkb^{F/F}$ mice. **e**, Intestinal inflammation detected by haematoxylin/eosin staining of caecal sections isolated from infected $Ikkb^{F/F}$ or $Ikkb^{\Delta IEC}$ mice on day 29 after infection. Scale bar, 50 μm. **f**, Serum LPS levels were determined by *Limulus* amoebocyte lysis (LAL) assay on samples from naive and infected $Ikkb^{F/F}$ and $Ikkb^{\Delta IEC}$ mice on day 29 after infection. n.d., not detected. Asterisk, $P < 0.05$. Error bars indicate s.e.m. Data are representative of two to four independent experiments ($n = 4$–6).

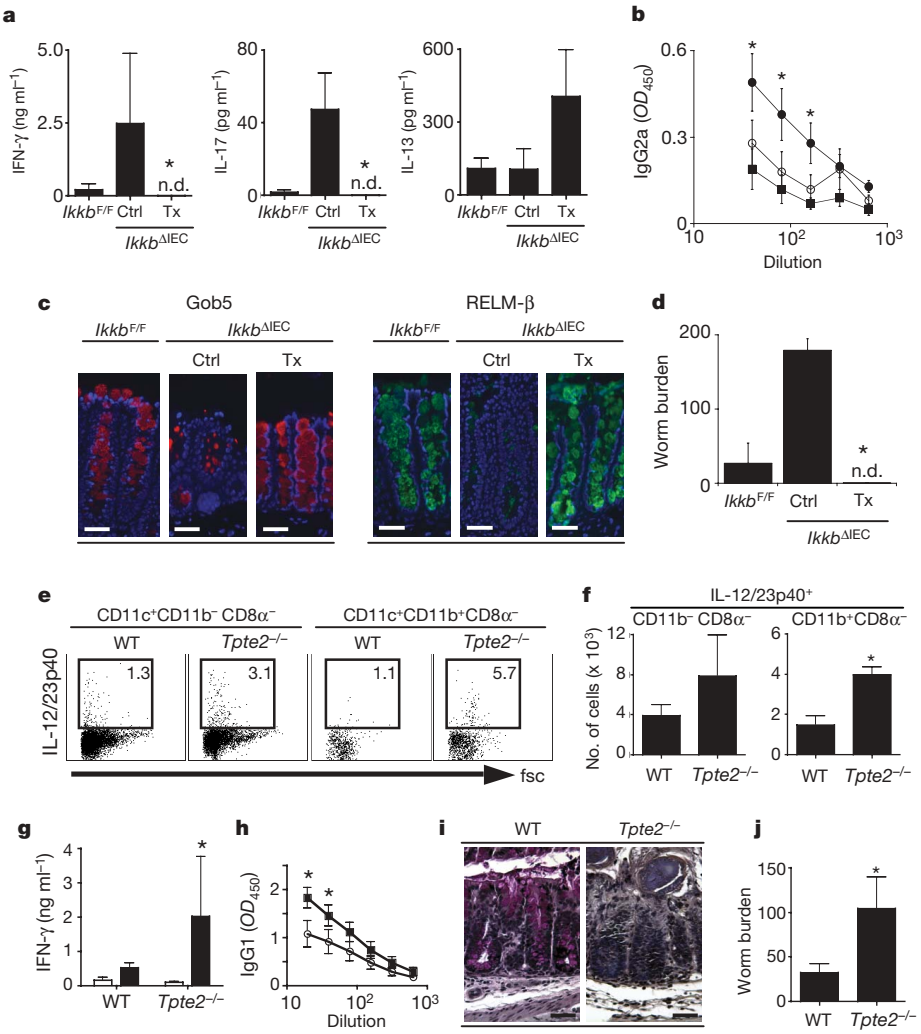

Figure 4 | IEC-intrinsic IKK-β is required for limiting proinflammatory cytokine responses in the GI tract. a, *Trichuris*-specific cytokine responses after restimulation of cells isolated from mLN of *Trichuris*-infected $Ikkb^{F/F}$ mice, infected $Ikkb^{\Delta IEC}$ mice treated with control immunoglobulin (Ctrl), and infected $Ikkb^{\Delta IEC}$ mice treated with monoclonal antibodies against IL-12/IL-23p40 and IFN-γ (Tx) mice. **b**, *Trichuris*-specific serum IgG2a (filled squares, infected $Ikkb^{F/F}$ mice; filled circles, infected $Ikkb^{\Delta IEC}$ mice; open circles, infected $Ikkb^{\Delta IEC}$ Tx mice). **c**, Gob5 or RELM-β expression in the caecum of infected $Ikkb^{F/F}$, $Ikkb^{\Delta IEC}$ and $Ikkb^{\Delta IEC}$ Tx mice on day 19 after infection. **d**, Worm burdens at day 19 after infection. n.d., not detected. **e**, DC responses in the caecal patch of wild-type (WT) and $Tpte2^{-/-}$ mice. **f**, Absolute number of IL-12/23p40^{+} DC subsets in the mLN of naive WT and $Tpte2^{-/-}$ mice. **g**, *Trichuris*-specific cytokine responses from infected WT and $Tpte2^{-/-}$ mice. Open bars, medium; filled bars, antigen. **h**, *Trichuris*-specific serum IgG1 levels (squares, WT; circles, $Tpte2^{-/-}$). **i**, Goblet cell hyperplasia in the caecum of infected WT and $Tpte2^{-/-}$ mice. Bar, 50 μm. **j**, Worm burdens at day 21 after infection. Asterisk, $P < 0.05$. Error bars indicate s.e.m. Data are representative of two experiments ($n = 4$–8).

$Tpte2^{-/-}$ mice were susceptible to infection with *Trichuris*, failing to expel worms as rapidly as wild-type mice (Fig. 4j). Thus, TSLP–TSLPR interactions are crucial for the development of protective type 2 immunity against *Trichuris*.

Taken together, these studies reveal a previously unrecognized role of IEC-derived factors in the regulation of intestinal immune homeostasis after exposure to a pathogen of the GI tract. Specifically, in addition to established pathways involved in pathogen recognition and the initiation of immune responses in the GI tract, which include M cells and specialized DC subsets that directly sample the luminal environment[27,28], results presented here support a role for IECs in directly influencing innate and adaptive immunity in the gut. Harnessing IKK-β-dependent functions of IECs will be an important consideration in the design and delivery of both anti-inflammatory agents and vaccines that target mucosal sites.

METHODS

Mice, parasites, antigens and infections. Animals were maintained in a specific-pathogen-free environment and tested negative for pathogens in routine screening. All experiments were performed at the University of Pennsylvania and University of California at San Diego in accordance with institutional guidelines. Mice were infected with 150–200 *Trichuris* eggs.

Cell culture, cytokine analysis and flow cytometry. Cells from naive or infected mice were plated in medium alone or in the presence of *T. muris* ES Ag (50 μg ml^{-1}), caecal bacterial antigen (100 μg ml^{-1}), LPS (10 ng ml^{-1}) or anti-CD3/CD28 (1 μg ml^{-1}; eBioscience). In some cases mLN cells were labelled with carboxyfluorescein succinimidyl ester (Molecular Probes) before stimulation. Cell-free supernatants were harvested and analysed for cytokine secretion by sandwich enzyme-linked immunosorbent assay (ELISA) or for intracellular cytokines by flow cytometry. Bone-marrow-derived DCs were conditioned for 16 h in the presence or absence of IEC-conditioned medium (CMT-93 cells) or TSLP (100 ng ml^{-1}; R&D Systems), before stimulation. IECs and LPLs were isolated and analysed *ex vivo* by flow cytometry. Gene expression was analysed by real-time polymerase chain reaction with the use of SYBR Green chemistry.

Analysis of IKK-β expression and NF-κB activation. Whole-cell extracts from IECs of naive or infected mice were analysed by SDS–polyacrylamide gel electrophoresis (PAGE) followed by immunoblotting with anti-IKK-β antibody (Cell Signalling). Extracts were analysed for DNA binding by ELISA (Pierce) or electrophoretic mobility-shift assay.

Serum immunoglobulin and LPS analysis. Serum was analysed for *Trichuris*-specific IgG1 and IgG2a by ELISA and for LPS by *Limulus* amoebocyte lysis assay (Sigma).

Goblet cell and RELM-β responses. For the detection of intestinal goblet cells, paraffin-embedded sections were stained with Alcian blue–periodic acid Schiff's reagent. Equal amounts of protein isolated from faecal pellets (20 μg) were analysed by SDS–PAGE and immunoblotted for RELM-β with a polyclonal rabbit anti-murine RELM-β antibody (Peprotech).

Statistical analysis. Results are shown as means ± s.e.m. for individual animals or conditions. Statistical significance was determined by Student's *t*-test (when comparing two groups) or analysis of variance with a post hoc test (when comparing more than two groups). Results were considered significant at $P < 0.05$.

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1. Nagler-Anderson, C. Man the barrier! Strategic defences in the intestinal mucosa. *Nature Rev. Immunol.* **1,** 59–67 (2001).
2. Neish, A. S. *et al.* Prokaryotic regulation of epithelial responses by inhibition of IκBα ubiquitination. *Science* **289,** 1560–1563 (2000).
3. Rimoldi, M. *et al.* Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nature Immunol.* **6,** 507–514 (2005).
4. Karin, M., Lawrence, T. & Nizet, V. Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. *Cell* **124,** 823–835 (2006).
5. 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).
6. Bethony, J. *et al.* Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet* **367,** 1521–1532 (2006).
7. Cliffe, L. J. & Grencis, R. K. The *Trichuris muris* system: a paradigm of resistance and susceptibility to intestinal nematode infection. *Adv. Parasitol.* **57,** 255–307 (2004).
8. Owyang, A. M. *et al.* Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. *J. Exp. Med.* **203,** 843–849 (2006).
9. Schopf, L. R., Hoffmann, K. F., Cheever, A. W., Urban, J. F. Jr & Wynn, T. A. IL-10 is critical for host resistance and survival during gastrointestinal helminth infection. *J. Immunol.* **168,** 2383–2392 (2002).
10. Artis, D. *et al.* Differential requirement for NF-κB family members in control of helminth infection and intestinal inflammation. *J. Immunol.* **169,** 4481–4487 (2002).
11. Tilney, L. G., Connelly, P. S., Guild, G. M., Vranich, K. A. & Artis, D. Adaptation of a nematode parasite to living within the mammalian epithelium. *J. Exp. Zoolog. A Comp. Exp. Biol.* **303,** 927–945 (2005).
12. Ghosh, S. & Karin, M. Missing pieces in the NF-κB puzzle. *Cell* **109** (Suppl.) S81–S96 (2002).
13. Li, Z. W., Omori, S. A., Labuda, T., Karin, M. & Rickert, R. C. IKKβ is required for peripheral B cell survival and proliferation. *J. Immunol.* **170,** 4630–4637 (2003).
14. Egan, L. J. *et al.* IκB-kinaseβ-dependent NF-κB activation provides radioprotection to the intestinal epithelium. *Proc. Natl Acad. Sci. USA* **101,** 2452–2457 (2004).
15. Greten, F. R. *et al.* IKKβ links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* **118,** 285–296 (2004).
16. Chen, L. W. *et al.* The two faces of IKK and NF-κB inhibition: prevention of systemic inflammation but increased local injury following intestinal ischemia-reperfusion. *Nature Med.* **9,** 575–581 (2003).
17. Artis, D. *et al.* RELMβ/FIZZ2 is a goblet cell-specific immune-effector molecule in the gastrointestinal tract. *Proc. Natl Acad. Sci. USA* **101,** 13596–13600 (2004).
18. Zhou, Y. *et al.* Characterization of a calcium-activated chloride channel as a shared target of Th2 cytokine pathways and its potential involvement in asthma. *Am. J. Respir. Cell Mol. Biol.* **25,** 486–491 (2001).
19. Kelsall, B. L. & Leon, F. Involvement of intestinal dendritic cells in oral tolerance, immunity to pathogens, and inflammatory bowel disease. *Immunol. Rev.* **206,** 132–148 (2005).
20. Rescigno, M. *et al.* Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nature Immunol.* **2,** 361–367 (2001).
21. Iwasaki, A. & Kelsall, B. L. Localization of distinct Peyer's patch dendritic cell subsets and their recruitment by chemokines macrophage inflammatory protein (MIP)-3α, MIP-3β, and secondary lymphoid organ chemokine. *J. Exp. Med.* **191,** 1381–1394 (2000).
22. Iwasaki, A. & Kelsall, B. L. Unique functions of $CD11b^+$, $CD8\alpha^+$, and double-negative Peyer's patch dendritic cells. *J. Immunol.* **166,** 4884–4890 (2001).
23. Li, M. *et al.* Topical vitamin D3 and low-calcemic analogs induce thymic stromal lymphopoietin in mouse keratinocytes and trigger an atopic dermatitis. *Proc. Natl Acad. Sci. USA* **103,** 11736–11741 (2006).
24. May, M. J. *et al.* Selective inhibition of NF-κB activation by a peptide that blocks the interaction of NEMO with the IκB kinase complex. *Science* **289,** 1550–1554 (2000).
25. Ziegler, S. F. & Liu, Y. J. Thymic stromal lymphopoietin in normal and pathogenic T cell development and function. *Nature Immunol.* **7,** 709–714 (2006).
26. Weaver, C. T., Harrington, L. E., Mangan, P. R., Gavrieli, M. & Murphy, K. M. Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity* **24,** 677–688 (2006).
27. Niess, J. H. *et al.* CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* **307,** 254–258 (2005).
28. Jang, M. H. *et al.* Intestinal villous M cells: an antigen entry site in the mucosal epithelium. *Proc. Natl Acad. Sci. USA* **101,** 6110–6115 (2004).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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