Cell

Exogenous Stimuli Maintain Intraepithelial Lymphocytes via Aryl Hydrocarbon Receptor Activation

Ying Li,[1] Silvia Innocentin,[4] David R. Withers,[5] Natalie A. Roberts,[5,6] Alec R. Gallagher,[2] Elena F. Grigorieva,[3] Christoph Wilhelm,[1] and Marc Veldhoen[1,4,*]
[1]Division of Molecular Immunology
[2]Division of Biological Services
[3]Division of Developmental Neurobiology
MRC National Institute for Medical Research, Mill Hill, London NW7 1AA, UK
[4]Laboratory of Lymphocyte Signalling and Development, Babraham Institute, Cambridge CB22 3AT, UK
[5]MRC Centre for Immune Regulation, Institute for Biomedical Research, Medical School, University of Birmingham, Birmingham B15 2TT, UK
[6]Present address: Department of Molecular, Cell and Developmental Biology, Yale University, New Haven, CT 06520, USA
*Correspondence: marc.veldhoen@babraham.ac.uk
DOI 10.1016/j.cell.2011.09.025

SUMMARY

The body's surfaces form the interface with the external environment, protecting the host. These epithelial barriers are also colonized by a controlled diversity of microorganisms, disturbances of which can give rise to disease. Specialized intraepithelial lymphocytes (IELs), which reside at these sites, are important as a first line of defense as well as in epithelial barrier organization and wound repair. We show here that the aryl hydrocarbon receptor (AhR) is a crucial regulator in maintaining IEL numbers in both the skin and the intestine. In the intestine, AhR deficiency or the lack of AhR ligands compromises the maintenance of IELs and the control of the microbial load and composition, resulting in heightened immune activation and increased vulnerability to epithelial damage. AhR activity can be regulated by dietary components, such as those present in cruciferous vegetables, providing a mechanistic link between dietary compounds, the intestinal immune system, and the microbiota.

INTRODUCTION

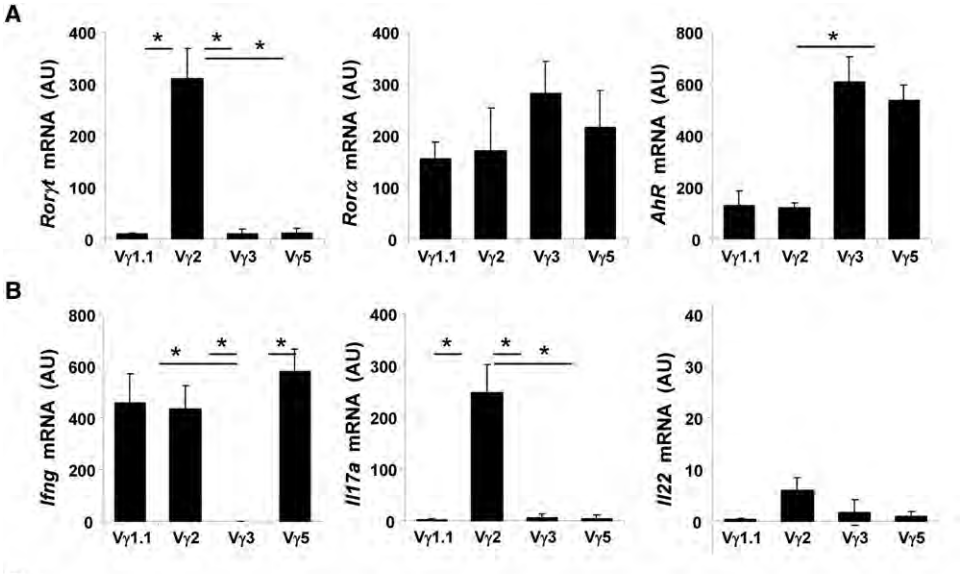
Our surfaces are colonized with a large variety of microorganisms. In the intestine, many of these aid in nutrient processing, immunity, tissue development, and provision of metabolic compounds. Although many microorganisms are beneficial, the tissues need protection against assault from these and others. A first line of defense is the physical obstruction provided by a single- or multi-cell layer, the epithelial barrier. In the skin this is a tight, although not impregnable, seal. In contrast, the epithelial cells of the intestine form leaky barriers required for supporting the exchange of nutrients and fluids. Underneath the epithelial barrier there is a network of immune cells, predominantly consisting of specialized intraepithelial lymphocytes (IELs). IELs are comprised of a distinct population of T cell receptor (TCR)$\gamma\delta$ cells in the skin and TCR$\gamma\delta$ and TCR$\alpha\beta$CD8$\alpha\alpha^+$ cells in the gut. They populate these sites before birth in preparation for subsequent colonization with microorganisms (Carding et al., 1990).

We recently reported the induced expression of the transcription factor aryl hydrocarbon receptor (AhR) in differentiated TCR$\alpha\beta^+$CD4$^+$ T helper (Th) 17 cells (Veldhoen et al., 2008), which is mirrored in populations of CD44^{hi}CCR6$^+$ TCR$\gamma\delta$ T cells found in secondary lymphoid organs (Martin et al., 2009). However, specialized TCR$\gamma\delta$ T cells found at epithelial sites, which predominantly display an alert phenotype without full activation (CD44^{int}CD25$^-$CD69$^+$CCR6$^-$), were not analyzed. In this study, we sought to determine whether these IELs express Th17-related factors that may play a role in barrier immunity. We report here that IELs express high levels of the AhR. Although the absence of AhR has no effect on the number or composition of general lymphoid cell populations, IELs have an intrinsic requirement for AhR activity. In the absence of AhR, IELs are not impaired in their ability to develop, home to their target organ, or proliferate. However, they are no longer maintained at the epithelial sites, in both the skin and the intestine. We reveal that intestinal AhR ligands are predominantly diet derived, with high levels present in cruciferous vegetables. The absence of AhR activation reduces epithelial cell turnover and the ability to control the microbial load and composition. As a consequence of low AhR activity, the intestine is in a heightened state of immune activation, dominated by a type 1 response, and more susceptible to immunopathology.

RESULTS

High Basal AhR Expression in IELs

IELs expressing an invariant TCR consisting of TCRVγ3 and TCRVδ1 receptor chains (see Garman et al., 1986 for



C

	Anatomical location	*Rorα*	*Rorγt*	*AhR*	*Gata3*	*Tbx21*	*Foxp3*	IFNγ	IL-17	IL-22
TCRVγ1.1	Systemic	++	_	+	++++	++	_	+	_	_
TCRVγ2	Systemic	++	++	+	++++	++	_	+	++	++(*)
TCRVγ3	Epidermis	++	_	+++	++++	+	_	_	_	_
TCRVγ5	Intestine	++	_	+++	++++	++	_	+/-	_	_

(*) upon AhR ligation

Figure 1. Gene Expression Profiles of Ex Vivo TCRγδ Subpopulations

(A and B) Average relative mRNA transcript levels to *Hprt* of indicated transcription factors (A) and cytokines (B) in FACS-purified populations of TCRγδ T cells (n = 5) identified by their TCRVγ usage.

(C) Overview table of TCRγδ subsets and their ex vivo gene profiles.

Data represent averages ± standard deviation (SD). See also Figure S1.

nomenclature) are among the first wave of T lymphocytes to mature in the embryonic thymus and populate the epidermis during keratinocyte stratification. By contrast, intestinal TCRγδ$^+$ cells develop in the thymus as well as extrathymically and mainly express TCRVγ5 determinants that can pair with multiple TCRVδ chains (Rocha et al., 1994). We compared the gene expression profiles of fluorescence-activated cell sorting (FACS)-sorted skin-derived TCRVγ3, intestinal TCRVγ5, and lymph node-sourced populations of TCRVγ1.1 and TCRVγ2 cells from nonimmune mice housed under specific pathogen-free conditions. We note a high basal ex vivo expression of AhR in both IEL subsets without additional cell activation. AhR expression significantly exceeded that found in TCRVγ2 and TCRVγ1.1 cell populations (which contain both naive and previously activated cells) (Figure 1A), differentiated Th17 cells, and hepatocytes (Veldhoen et al., 2008). This is in agreement with an earlier study that noted differential expression of AhR in intestinal IELs compared with lymph node-sourced TCRαβ$^+$CD8αβ$^+$ T cells as well as intestinal epithelial cells (Fahrer et al., 2001). We confirmed that expression of the Th17 cell transcriptional regulator, the orphan nuclear receptor *RORγt*, was enriched in TCRVγ2$^+$ cells (Martin et al., 2009), whereas all TCRγδ subsets expressed the closely related factor *RORα* (Figure 1A). Other mRNA levels for relevant Th transcription factors are tabulated (Figure 1C; Figure S1A available online).

In confirmation of their state of ''alertness'' but not full activation, ex vivo interferon (IFN)-γ transcripts were very low in TCRVγ3$^+$ cells (Figures 1B and S1B), and only low amounts could be detected in the TCRVγ5$^+$ intestinal IEL fraction (Figures 1B and S1C). Consistent with the absence of *RORγt* (Figure 1A), IL-17 transcripts and protein were absent in IELs (Figures 1B, S1B, and S1C). This was in stark contrast with TCRγδ cells found in the dermis, which displayed an activated (CD44^{hi}CD25$^+$ CCR6$^+$) phenotype and of which up to 50% readily produced IL-17 (Figure S1B). Despite high basal levels of AhR transcripts, no IL-22 transcripts or protein could be detected in ex vivo TCRVγ3$^+$ and TCRVγ5$^+$ cells (Figures 1B, S1B, and S1C), concordant with our earlier finding that AhR may be required but alone is not sufficient for IL-22 production (Veldhoen et al., 2009).

AhR Deficiency Results in the Specific Loss of IELs

The basal expression of AhR in IEL populations led us to explore the consequences of the loss of AhR. Mice deficient in one or both AhR alleles showed no significant changes in the proportions or numbers of TCR$\gamma\delta$ T cell subsets in lymph nodes, spleen, or thymus (Figures 2A, 2C, and S1D). However, there was a striking loss of over 95% of TCR$\gamma\delta$ cells in the small intestine in the absence of AhR compared with controls, without bias toward Thy1 expression levels (Figures 2A, 2C, and 2F). Furthermore, skin isolates from AhR-deficient mice, containing both dermis and epidermis, showed a distinctly different TCR$\gamma\delta$ profile with the absence of TCR$\gamma\delta^{hi}$ cells (see arrow, Figure 2A). TCRVγ3-specific staining positively identified these cells, highlighting the absence of this epidermis-specific subset in AhR-deficient mice (Figures 2B, 2D, and 2G). Numbers of CD4$^+$ and CD8$\alpha\beta^+$TCR$\alpha\beta^+$ T cells were similar in both mouse strains (Figures S2A and S2B), as previously reported (Schmidt et al., 1996). However, TCR$\alpha\beta^+$CD8$\alpha\alpha^+$ T cells, the other major population of intestinal IELs with elevated AhR expression (Figure S2C), are also significantly reduced in AhR-deficient mice (Figures 2E and S2D). By contrast, the proportions (and numbers) of regulatory T cells (Tregs), CD4$^+$ lymphoid tissue inducer (LTi)-like cells, or natural killer (NK) p46$^+$ NK-like cells in the lamina propria (LP) of the intestine or spleen were not significantly altered in AhR-deficient mice compared with controls (Figures S2E–S2G). Although the absence of TCRVγ3$^+$ cells in AhR-deficient mice dramatically changed the proportional representation of TCR$\gamma\delta$ T cells in the skin, in the intestine the relative distribution of TCRVγ chains followed that of control mice, dominated by TCRVγ5$^+$ IELs (Figure S1D). In situ staining for TCR$\gamma\delta$ confirmed these findings and illustrates the close spatial relationship of IELs with epithelial cells in the intestine and the dense network of cells in the epidermis (Figures 2F and 2G).

IELs Develop in the Absence of AhR

AhR deficiency has been linked with altered development of peripheral lymphocytes in one of the three differently gene-targeted AhR-deficient mouse strains, albeit not in the other two (Lahvis and Bradfield, 1998; Schmidt et al., 1996). Furthermore, exposure to the nonbiodegradable AhR ligand 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) may contribute to thymic involution (Knutson and Poland, 1982). This raises the possibility that the selective reduction in IELs observed in AhR-deficient hosts could have a developmental origin. However, we did not observe proportional or numerical differences of TCRVγ3 cells in the embryonic thymus between AhR-sufficient and -deficient mice (Figure S3A). In addition, even if TCRVγ3 cells are reduced to 10% of fetal thymocytes, they still predominate in the adult epidermis (Xiong et al., 2002). Furthermore, TCRVγ3$^+$ T cells did home to the skin in the absence of AhR, as they were detectable in neonates and 5-week-old mice, but they were lost over time (Figures S3B, S3C, 2A, and 2G). We did not encounter TCRVγ3$^+$ cells at other anatomical sites examined, including the dermis and peritoneum, excluding aberrant homing properties in the absence of AhR. TCRVγ5$^+$ T cells, which can be generated in the adult thymus, could also be detected in the adult thymus as well as in peripheral lymphoid organs of AhR-deficient mice, in ratios and absolute numbers similar to those of control mice (Figure S1D and data not shown). This is consistent with the hypothesis that the generation and migration of epithelial TCR$\gamma\delta$ T cell subsets proceeds similarly in AhR-deficient hosts compared to controls, but that their maintenance at mucosal sites is compromised in the absence of AhR-dependent signals.

Loss of IELs in the Absence of AhR Is Cell Intrinsic

As AhR is expressed in many tissues, we determined whether IEL-intrinsic or -extrinsic mechanisms were responsible for the specific reduction in IELs. Adoptive transfers of FACS-purified AhR-sufficient TCR$\gamma\delta$ T cells, from secondary lymphoid organs or intestine, only effectively reconstituted in the small intestinal epithelia of AhR-deficient but not control mice (data not shown). This is consistent with the absence of an endogenous IEL population in AhR-deficient mice, providing a niche for the transferred cells. Chimeras generated by transfer of bone marrow cells from control mice reconstituted the intestinal IEL populations in recombinase-activated gene (Rag) 1-deficient hosts even when the hosts were Rag1 and AhR double-deficient (Figures S3D and S3E). Although AhR-deficient bone marrow successfully reconstituted secondary lymphoid organs as well as TCR$\alpha\beta^+$ cells in organs such as liver and intestine (data not shown), it failed to reconstitute intestinal IELs (Figure S3D).

To distinguish between potential AhR-dependent activity in bone marrow-derived lymphoid or accessory cells, we made use of mice with a floxed AhR allele (Walisser et al., 2005) and introduced Cre recombinase under the promoter of the *Rag1* gene, allowing lymphocyte-specific deletion of AhR. In these mice, the defect in intestinal IELs (Figure 3A) and TCRVγ3 (TCR$\gamma\delta^{hi}$CCR6$^-$) cells in the skin was recapitulated (Figure 3B) and indistinguishable from that in germline AhR-deficient mice (Figure 2). The collective data obtained support the view that the maintenance of IELs depends critically on T cell-intrinsic AhR activity.

IELs Proliferate in the Absence of AhR

AhR-derived signals have been suggested to modulate a broad array of genes, including factors that play a role in cell-cycle regulation and cell death (Boitano et al., 2010; Marlowe and Puga, 2005). To investigate the contribution of AhR-dependent proliferative and cell survival signals in IELs, we assessed their proliferative capacity by incorporation of 5-ethynyl-2′-deoxyuridine (EdU) in vivo. Two days of EdU administration showed a significantly increased proliferative capacity of AhR-deficient IELs compared with controls (Figures 3C and S3F). However, 4 days of administration resulted in a similar proportion of IELs that incorporated EdU in both groups of mice. Furthermore, termination of EdU administration resulted in a rapid contraction of EdU$^+$ IELs in AhR-deficient mice compared with controls (Figures 3C and S3F). These results indicate that the proliferative capacity of IELs is not affected by the absence of AhR, but that their reduced survival potential is the likely cause of their diminished numeric presence.

Intestinal AhR Ligands Are Diet Derived

AhR contains two highly conserved, period clock-AhR nuclear transporter (Arnt)-single-minded (PAS) domains. Phylogenetic analysis indicates that PAS domain-containing proteins have

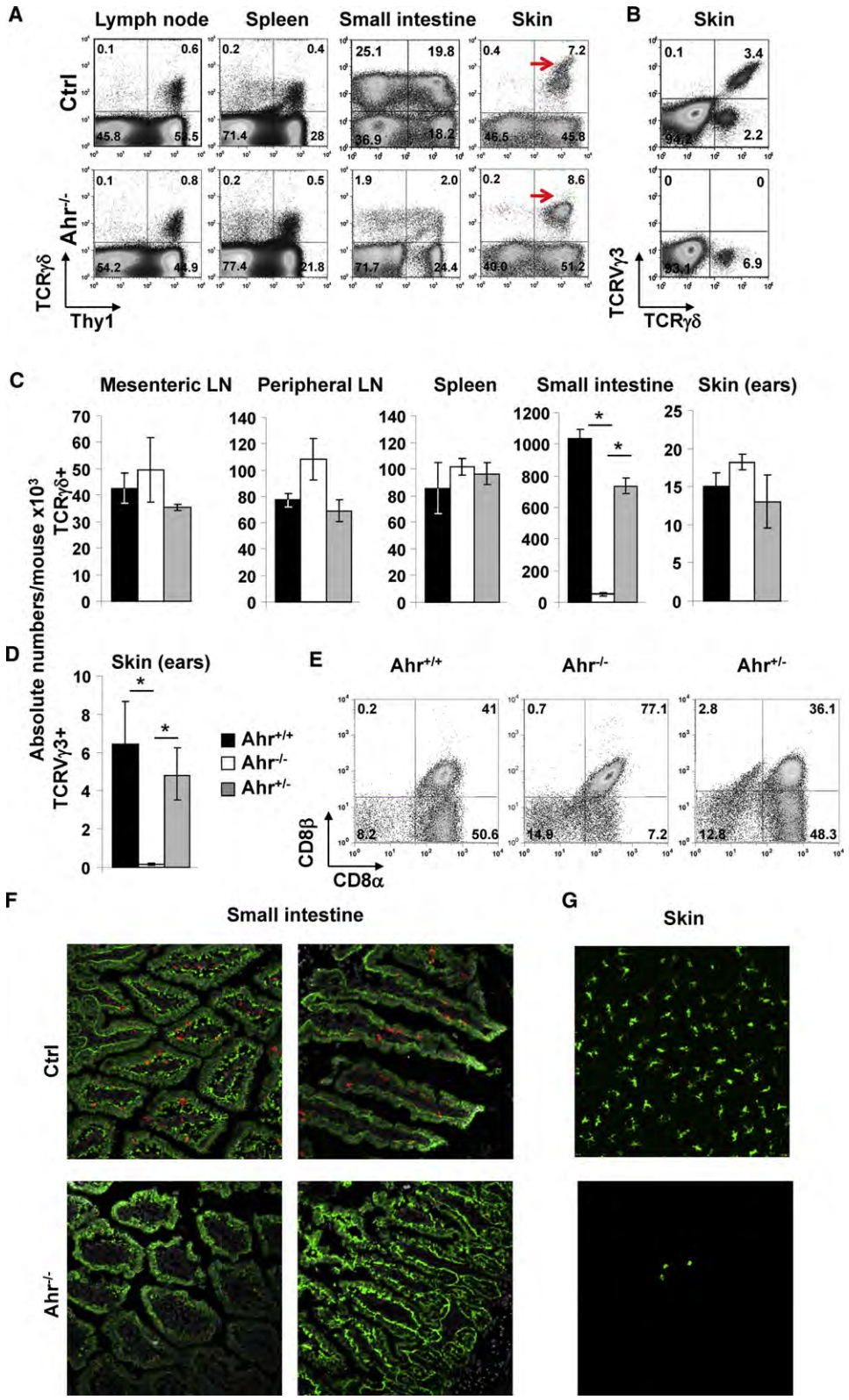


Figure 2. Specific Reduction of IELs in the Absence of AhR

(A–D) Analysis of TCR$\gamma\delta$ T cell subpopulations, identified by their TCRVγ usage, present in indicated tissues in control (upper panels) and AhR-deficient (lower panels) mice analyzed by FACS (n > 10) (A and B) and absolute numbers (n = 8) (C and D).

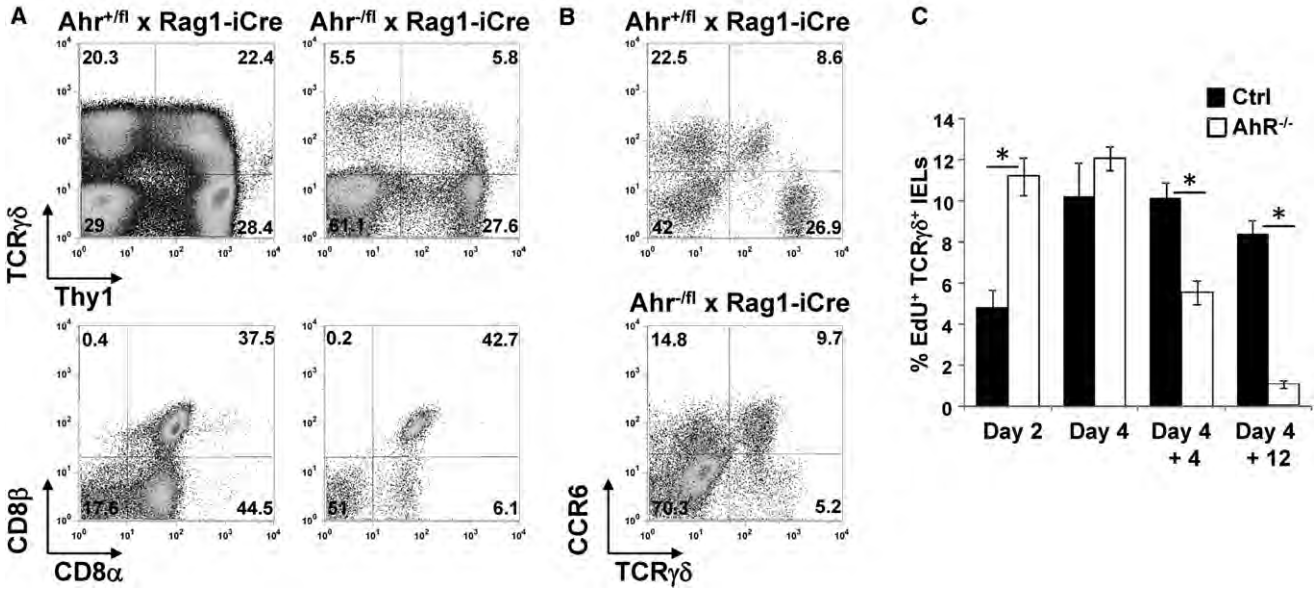


Figure 3. IEL Maintenance Depends on Intrinsic AhR Activity

(A and B) Representative FACS plots (n > 6) of control mice or mice with targeted AhR deficiency in Rag1-expressing cells in the intestine, staining for TCR$\gamma\delta^+$ (upper panels) or pregated TCR$\alpha\beta^+$ (bottom panel) cells (A), and skin, revealing TCRVγ3 cells via staining for TCR$\gamma\delta$ and CCR6 (B).

(C) Percentage of proliferating cells detected by EdU incorporation at indicated time points and after (+) termination of EdU administration in control (■) and AhR-deficient (□) mice (n = 4).

Data represent averages ± SD. See also Figure S3.

primarily evolved to respond to environmental changes in energy (Taylor and Zhulin, 1999). The PAS domains in AhR/Arnt closely resemble those present in photoactive yellow protein (PYP) (Pellequer et al., 1998). Analogous to PYP, AhR is responsive to the tryptophan photoproducts 6-formylindolo [3,2-b] carbazole (FICZ) and 6,12-diformylindolo[3,2-b] carbazole (dFICZ) (Oberg et al., 2005), and AhR-dependent *Cyp1a1* expression can be found in the skin in the absence of exogenous xenobiotics (Sadek and Allen-Hoffmann, 1994). However, the equivalent to mouse TCRVγ3 IELs are not present in humans due to the absence of *skint1*(Boyden et al., 2008), and further studies were concentrated on intestinal IELs.

Expression of the AhR target gene *Cyp1a1* in the intestine has been shown to depend directly on dietary AhR ligands (Ito et al., 2007). Indeed, we observed AhR-dependent Cyp1a1 expression (Figure 4A). The chemical complexity of the diet makes it difficult to determine the exact nature of all potential AhR ligands. However, the tryptophan-derived phytochemical I3C, found in cruciferous vegetables, can be converted into the high-affinity AhR ligands indolo[3,2-b]carbazole (ICZ) and 3,3-diindolylmethane (DIM) (Bjeldanes et al., 1991). In order to evaluate a dietary source of AhR ligands, we fed control C57BL/6 mice either standard diet (5021-3 Autoclavable Rodent Lab Diet) or a synthetic purified diet (AIN-76A Purified Rodent Diet). Exposure of control mice for 3 weeks to the purified synthetic diet significantly reduced *Cyp1a1* expression in the ileum compared to standard diet (Figure 4A). Supplementation of AIN-76A with 200 ppm I3C was able to induce a higher level of *Cyp1a1* transcripts than our standard diet (Figure 4A). In line with our hypothesis that AhR expression is required for maintaining IELs, we found a significant decrease in both TCR$\gamma\delta^+$ and TCR$\alpha\beta^+$CD8$\alpha\alpha^+$ IELs in the small intestine of mice fed with synthetic diet compared with standard diet (Figures 4B and S4A). Supplementing the same synthetic diet with only the AhR ligand precursor I3C restored both TCR$\gamma\delta$ and TCR$\alpha\beta$CD8$\alpha\alpha$ IELs to levels comparable to those with the standard diet (Figures 4B and S4A).

AhR Deficiency Results in an Increased Bacterial Burden

IELs share many properties with conventional TCR$\alpha\beta$ T cells (Fahrer et al., 2001), which suggests a degree of overlap in their functional properties. Two crucial differences are the capacity of IELs to respond swiftly, without the need for clonal expansion or priming, and their crosstalk with cells that make up the epithelial barriers. Consistent with the reduction of IELs in AhR-deficient mice, intestinal epithelial turnover was reduced compared with control mice (Figure S4B) (Boismenu and Havran, 1994; Komano et al., 1995). In line with this, adoptive transfer of

(E) FACS analysis of TCR$\alpha\beta^+$ lymphocytes in the IEL small intestinal fraction of control mice and those deficient for AhR at one or both alleles, showing relative contributions of CD8$\alpha\beta$ and CD8$\alpha\alpha$ populations.

(F and G) Tissue samples from control (upper panels) or AhR-deficient (lower panels) mice were stained with antibodies against EpCam (green) and TCR$\gamma\delta$ (red) in the small intestine (F) or TCRVγ3 (green) in the skin (mice aged 8 weeks) (G).

Figures are representative of at least four experiments. Data represent averages ± SD. See also Figure S2.

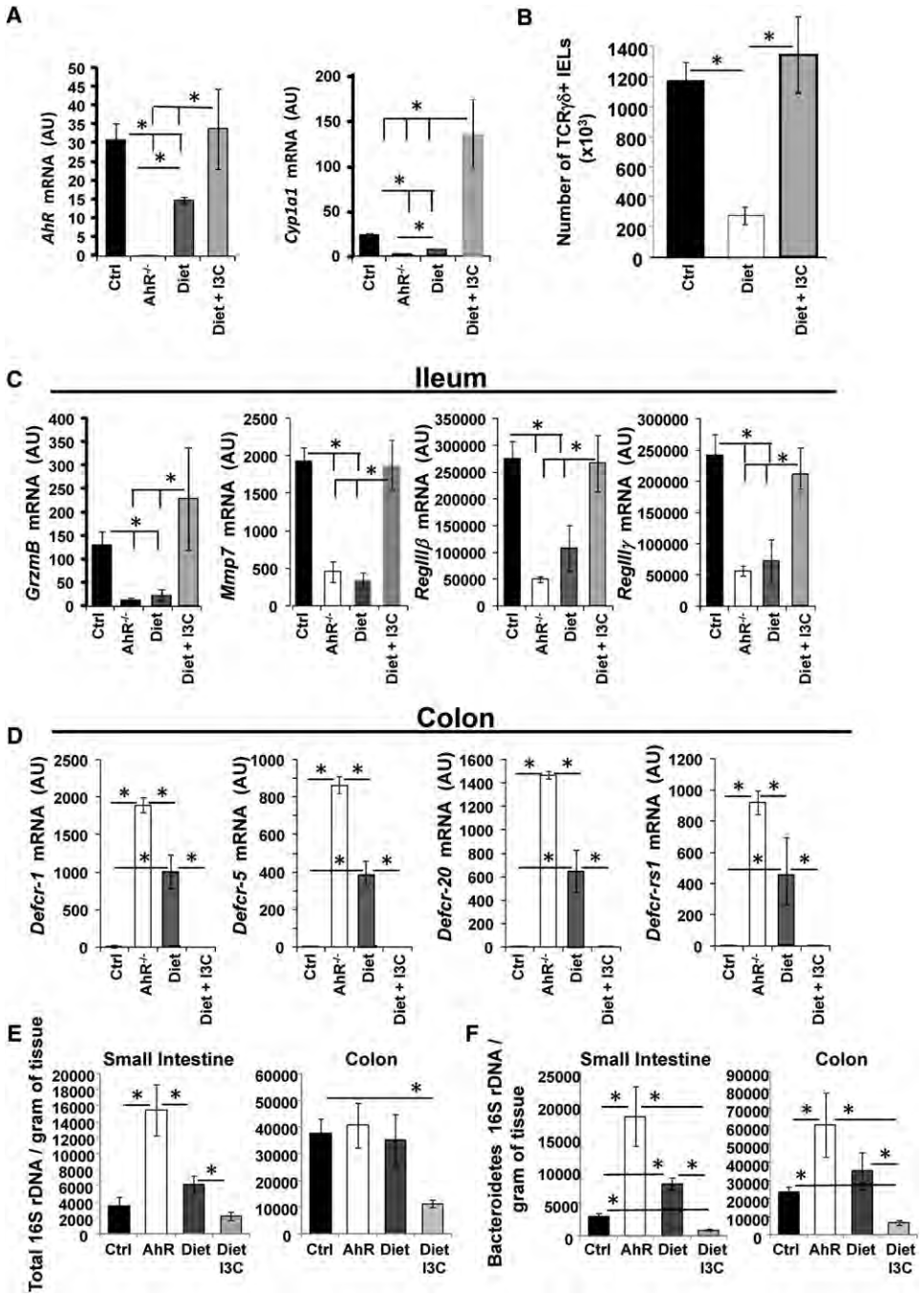


Figure 4. IEL Maintenance Depends on Dietary-Derived Ligands and Alters the Bacterial Load

(A) Relative mRNA transcript levels of AhR and Cyp1a1 in small intestine of control and AhR-deficient mice fed standard diet or control mice fed synthetic diet or synthetic diet supplemented with I3C (n = 5).

(B) Absolute numbers of small intestinal TCR$\gamma\delta^+$ IELs in mice fed standard diet (ctrl), synthetic diet, or synthetic diet supplemented with I3C for 3 weeks (n = 6).

(C and D) relative mRNA transcript levels of indicated genes (n = 5) in the ileum (C) and colon (D) in indicated experimental groups.

(E and F) Relative presence of total bacterial- (E) or Bacteroidetes-specific (F) S16 ribosomal DNA in indicated experimental groups (n = 8).

Data represent averages ± SD. See also Figure S4 and Table S1.

AhR-sufficient intestinal TCR$\gamma\delta^+$ lymphocytes into AhR-deficient recipients restored epithelial cell proliferation to a level seen in control mice.

TCR$\gamma\delta$ deficiency can result in more robust development of host immunity, often accompanied by increased immune pathology, implicating IELs in tolerance (Girardi et al., 2003). In

our colony of AhR-deficient mice bred under SPF conditions, we see no overt signs of ill health, but transfer of such mice to a less controlled environment increases the occurrence of rectal prolapse and signs of colitis in aging mice and of deaths in young mice due to severe ileitis. Other AhR-deficient colonies, experiencing different environments, were reported to suffer from skin abnormalities, rectal prolapse, and premature deaths (Fernandez-Salguero et al., 1995; Kimura et al., 2009). We hypothesized that the reduction in IELs could result in bacterial translocation into the sterile tissues due to increased barrier permeability, but we did not detect any bacteria in the colon-draining mesenteric lymph node, nor did we observe an increase in serum levels of LPS-binding protein (LBP) or increased intestinal permeability in healthy, AhR-deficient mice compared with controls after dextran-FITC feeding (Figure S4C and data not shown). Histological analyses of the small intestine did not reveal intestinal damage but did highlight increased villus length, epithelial cell size, and apical cytoplasmic mucin distention in AhR-deficient mice compared with controls (Figure S4D). This observation may be the result of decreased epithelial cell turnover in AhR-deficient mice and may contribute toward maintaining barrier integrity in the absence of IELs.

IELs are directly involved in immune surveillance, inducing apoptosis in infected cells via expression of high levels of granzymes (Fahrer et al., 2001). In line with the significant reduction of IELs in mice with reduced AhR stimulation, we detected significantly lower levels of granzyme A and B expression in the small intestine compared with control mice and those on a synthetic diet supplemented with I3C (Figure 4C and data not shown). This suggested that in the absence of AhR, there might be a reduction in the control of microorganisms, prompting the analysis of bactericidal gene expression in the gut. Although the small intestine did not show differential expression of the antimicrobial cryptidins tested (Figure S4E), the expression of matrix metalloproteinase (MMP)-7, which is required for wound healing and the bactericidal activity of cryptidins (Wilson et al., 1999), was significantly reduced in mice with reduced AhR activity compared with controls (Figure 4C). In addition, significant differences were found between controls and mice deficient in AhR or those maintained on a diet with low AhR ligands in the expression levels of the antimicrobial C-type lectins regenerating islet-derived protein 3 (RegIII)β and RegIIIγ (Figure 4C). These lectins are secreted into the intestinal lumen and enhance the clearance of Gram-positive bacteria (Abreu, 2010). The reduced expression of granzymes, C-type lectins, and MMP-7 suggests a diminished control of the intestinal microbial load in mice with reduced or absent AhR activity.

In line with this expectation, qPCR analysis of microbial-specific ribosomal 16S DNA revealed a 4-fold increase in bacterial load in the small intestine, but not the colons, of AhR-deficient mice compared with controls (Figure 4E). A reduced intake of dietary AhR ligands for 3 weeks increased the bacterial load, whereas supplementation of this diet with I3C reduced the microbial load compared with controls, especially in the colon. The contribution to the microbial make-up by the phyla Firmicutes, Actinomyces, or Proteobacteria was not significantly altered between the experimental groups (Figures S4G and S4H). The heightened bacterial load in the small intestine could be attributed to the increase in the phylum Bacteroidetes (Figure 4F), which contains members of the genus *Bacteroides*. Although largely mutualistic, *Bacteroides* contain many known opportunistic pathogens and have been implicated in a mouse model of inflammatory bowel diseases (IBD) (Bloom et al., 2011). Although the bacterial load in the colon was similar in all experimental groups, a proportional increase in the contribution of Bacteroidetes was also observed here in the context of reduced AhR activity, compared with controls (Figure 4F).

Expression of bactericidal C-type lectins, MMP-7, or granzymes was low in the colon compared with the small intestine in all groups of mice (Figure S4F). Cryptidin transcripts, however, although absent in the colons of control mice and of those on synthetic diet supplemented with I3C, could readily be detected at highly significant levels in the colons of AhR-deficient mice and of control mice on a synthetic AhR ligand-low diet (Figure 4D). In addition to this indication of a heightened state of immune activation, we found increased expression levels of IFN-γ, but not TGF-β1, in mice with no or low AhR activity compared with controls (Figure S5A). The synthetic diet, irrespective of the presence of I3C, induced significantly higher levels of IL-10 transcripts in the colon compared with our standard diet.

AhR Deficiency Increases Epithelial Immunopathology

The increased immune cell activation status prompted further analysis of the adaptive immune response in the colonic LP and IEL compartments. Lymphocyte analysis revealed an increased proportion and number of TCR$\alpha\beta^{+}$CD4^{+} and CD8^{+} cells in the colonic IEL fraction (Figure 5A), with those in both the IEL and LP compartments producing high levels of IFN-γ but little IL-17 (Figures 5A and S5C). Administration of the broad-spectrum antibiotic Enrofloxacin (Baytril, Bayer) was able to prevent both the numerical increase of T cells and their IFN-γ production (Figure 5A), illustrating the causative effect of the microbiota on immune activation in the absence of AhR activity.

In order to address the consequences of AhR deficiency for intestinal physiology, we employed dextran sodium sulfate (DSS)-induced colitis, a model of chemically induced epithelial damage and inflammation that is exacerbated by bacterial dissemination, especially in the absence of IELs (Ismail et al., 2009; Wirtz et al., 2007). Administration of 3% DSS resulted in rapid weight loss in control mice but full recovery after DSS withdrawal at day 6 (Figure 5B). However, in the absence of AhR, DSS caused accelerated weight loss, extreme shortening of the colon, and severe hemorrhaging, with 13 out of 16 mice reaching over 20% of body weight loss 1 day after DSS was withdrawn (Figures 5B and S5B). In agreement with the critical role of AhR, control mice fed with an AhR ligand-low diet for 4 weeks rapidly lost weight and showed a shortening of the colon similar to that in AhR-deficient mice, with 11 out of 16 mice losing over 20% of body weight (Figures 5C and S5B). However, mice fed the purified diet supplemented with I3C showed only mild signs of colitis, with reduced weight loss and shortening of the colon, followed by a rapid recovery post-DSS administration (Figures 5C and S5D). Reconstitution of AhR-deficient hosts with control IELs 8 weeks prior to 3% DSS administration did reduce the initial weight loss, but this was still increased compared with controls (Figure 5D). However, all of the AhR-deficient mice

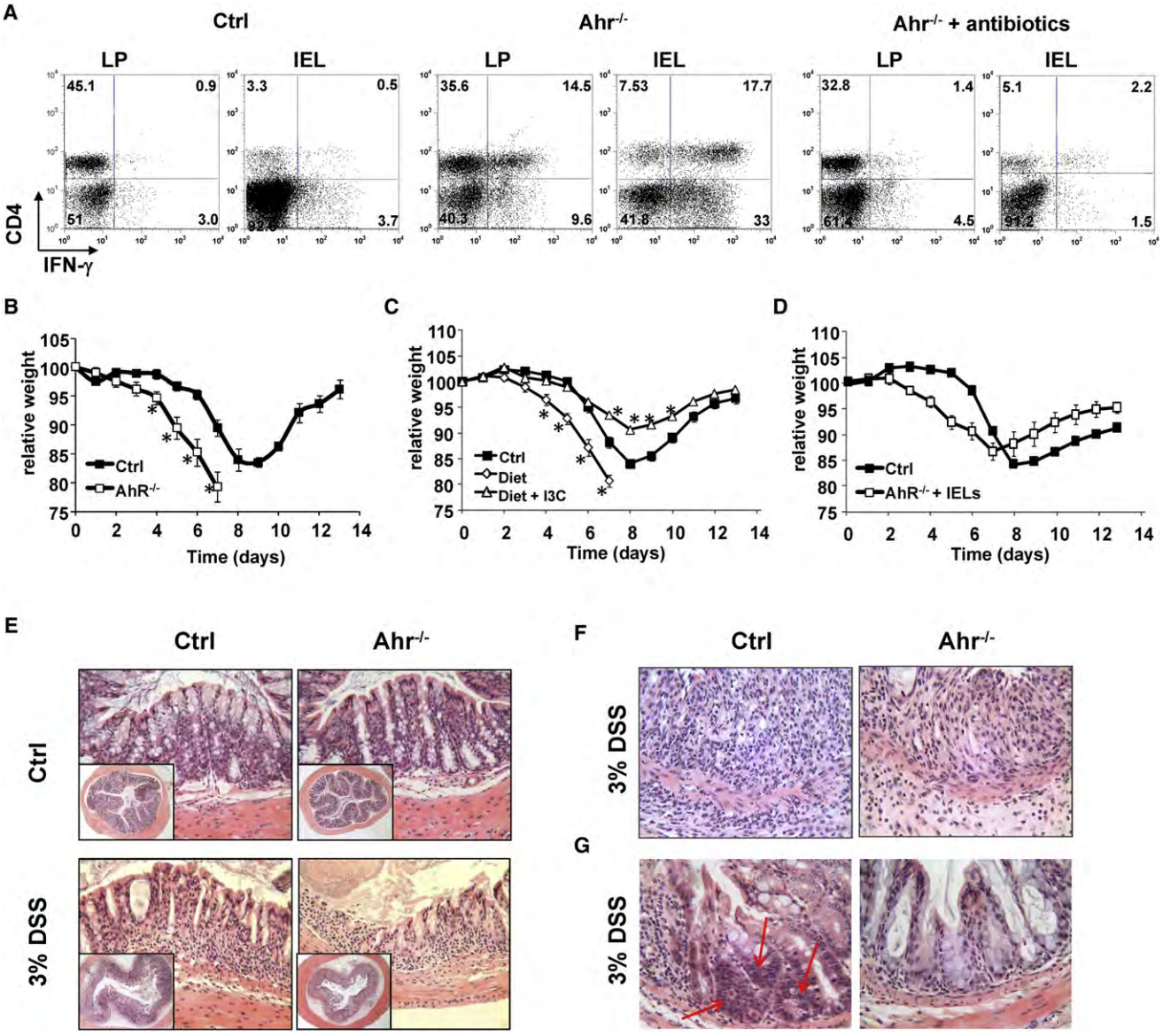


Figure 5. The Absence of AhR or Dietary AhR Ligands Increases the Severity of Colitis

(A) Representative FACS plots (n = 6–8) of cells harvested from the lamina propria (LP) or intraepithelial cell (IEL) fraction of the colon of control or AhR-deficient mice or the latter after antibiotic treatment, plotting CD4 against IFN-γ.

(B–D) Relative weights to starting weight of control (■) and AhR-deficient (□) mice after 3% DSS administration (n = 8) (B); or of control mice fed standard diet (■), AhR ligand-low diet (◇), or AhR ligand-low diet supplemented with I3C (△) (C); or of control (■) and AhR-deficient mice 8 weeks after adoptive transfer with control IELs (□) fed standard diet (D). n = 8 per group, representative of at least two biological repeats.

(E, F, and G) H&E staining of colons of untreated or 3% DSS-treated controls or AhR-deficient mice. (E) Images are 250×, insets are 65×, and (F) and (G) are 400× magnification. (G) Arrows indicate proliferating cells at the base of colonic crypts.

All figures are representative of at least six individual mice.Data represent averages ± standard error of the mean (SEM). See also Figure S5.

reconstituted with control IELs (16 out of 16) made a full recovery, emphasizing the importance of IELs in reducing DSS-induced colitis.

The severity of the tissue damage in AhR-deficient colons was illustrated in histological sections, showing a diffuse LP, increased destruction of colonic epithelium, but reduced immune cell infiltration (Figures 5E and 5F). Importantly, in control mice, colonic crypt stem cells retain the ability to proliferate (indicated by arrows) and replace the damaged epithelial cells. By contrast, this epithelial repair mechanism was not observed in AhR-deficient colons (Figure 5G). These data suggest that AhR activation by dietary compounds is required to maintain IEL populations, thereby reducing the susceptibility to intestinal pathology via increased microbial control. This results in reduced recruitment of T lymphocytes. In addition, increased microbial control reduces the bacterial load and the proportion of potential

pathogens thereby reducing bacterial dissemination upon epithelial damage. Furthermore, IELs enable rapid barrier repair with acute and local production of epithelial growth factors.

DISCUSSION

We provide experimental evidence that external environmental factors can have a direct and profound impact on the host's immune defense capacity. Barrier-resident immune cells, the IELs, found in the murine skin and the small intestine have a cell-intrinsic requirement for the ligand activation of the AhR. The IEL populations TCR$\alpha\beta$CD8$\alpha\alpha$ and TCR$\gamma\delta$ lymphocytes express high levels of AhR. Its activation directly affects the maintenance, but not the development, homing, or proliferation, of IELs. In addition, we show that cruciferous vegetables are an important source of AhR ligand(s) in the intestine. These vegetables are enriched in the breakdown product of glucosinolate glucobrassicin, I3C, which under the influence of stomach acids can be converted to the high-affinity AhR ligands DIM and ICZ.

Importantly, the same epithelial barrier sites that harbor IELs are also host to a diverse but tightly managed composition and amount of microorganisms, harboring species that contribute positively to health but also pathogenic and opportunistic ones that form a potential threat. We show that a reduction in AhR activity in mice reduces their intestinal cytotoxic capacity with a profound reduction in perforin and granzymes and reduces their capacity to express or activate antimicrobial proteins. Although the microbicidal action of these peptides is often considered mild, concentrations in the crypts can reach 10 mg/ml after microbial stimulation, sufficient for strong bacterial lysis (Ayabe et al., 2000). As a result, the bacterial load in mice with reduced AhR activity is increased, particularly associated with an enhanced contribution of species of the *Bacteroidetes* phylum.

An altered bacterial composition, but not load, in the absence of AhR activity was not confined to the small intestine, where the IEL populations are reduced, but was also observed in the colon. This results in an accumulation of lymphocytes that show a predominant type 1 profile characterized by the production of IFN-γ. This is in line with other murine models of colitis that report a deleterious role of the intestinal microbiota, especially during the transfer of T cells, and are dominated by a type 1 profile (Cong et al., 1998). The direct importance of the microbiota in the activation of T cells was illustrated when AhR-deficient mice were treated with a broad-spectrum antibiotic, reducing both the number of T cells and their activation status. Our data are in also line with observations made in mice genetically engineered to lack TCR$\gamma\delta$ lymphocytes, contributing over half of the intestinal IELs, which are more susceptible to certain bacterial, protozoal, and viral infections and show increased morbidity (King et al., 1999; Mombaerts et al., 1993; Moore et al., 2000; Roberts et al., 1996; Selin et al., 2001), and they are in agreement with increased morbidity of AhR-deficient mice and their increased susceptibility to colitis and rectal prolapse (Fernandez-Salguero et al., 1997; Kimura et al., 2009).

We show that AhR deficiency or the reduced intake of dietary AhR ligands contributes to increased immunopathology in a model of DSS-induced colitis, causing injury to colonic epithelial cells with enhanced immunopathology due to bacterial dissemination (Wirtz et al., 2007). Although reduced control of the microbial load or composition in mice deficient in critical microorganism control mechanisms results in increased susceptibility to DSS-induced injury and reduced epithelial proliferation (Rakoff-Nahoum et al., 2004; Vijay-Kumar et al., 2007), mice treated with broad-spectrum antibiotics or bred under germ-free conditions are similarly susceptible to DSS-induced colitis (Maslowski et al., 2009; Rakoff-Nahoum et al., 2004). This indicates that both elements of the microbiota and detection mechanisms are required for maintaining a healthy epithelial barrier (Mazmanian et al., 2008). Although IELs were important in reducing excessive DSS-induced colitis, the kinetics of initial accelerated weight loss after adoptive transfer of control IELs (Figure 5D) could indicate that other cell types may play an additional role. In line with this, we show that in the absence of IELs, approximately 50% of AhR transcripts can still be detected in the small intestine of mice fed an AhR ligand-low diet compared with controls (Figure 4A). It is possible that AhR, although not essential for the survival, development, or migration of other cell types, is important for their function, for example, for the induced production of IL-22 in Th17 cells or in populations of innate lymphoid cells (Cua and Tato, 2010; Veldhoen et al., 2008) that may influence recovery from DSS.

Whether AhR and its ligands affect human intestinal immunobiology is currently unknown. However, there are some observations of interest. Increased CD4-mediated IFN-γ production, epithelial damage, hyperplasia, apical cytoplasmic mucin distention, reduced α-defensin expression, and an increased bacterial load have all been associated with IBD (Fuss et al., 1996; Wehkamp et al., 2005). A body of data correlate individual components or alterations in the intestinal microbial composition in driving the mucosal inflammatory response in susceptible individuals suffering from IBD (Frank et al., 2007; Harper et al., 1985; Sokol et al., 2006). It is of note that a number of studies have also shown an association between high faecal or mucosal levels of *Bacteroides*, the genus of Bacteroidetes predominantly found in mammals, and active IBD (Andoh et al., 2011). Furthermore, lesions in IBD occur in those areas with highest bacterial exposure, and broad-spectrum antibiotics can be successfully used as primary therapy in uncomplicated IBD (Shanahan, 2000). Isolated bacterial species involved are nonpathogenic in healthy hosts, suggesting that effective regulatory mechanisms are normally in place to maintain intestinal homeostasis preventing inflammation. Epidemiological studies indicate a strong environmental influence on IBD, with a low concordance rate between identical twins and a link with a Western lifestyle (Loftus, 2004), a diet low in fruit and vegetables being one of the major risk factors (Amre et al., 2007; D'Souza et al., 2008; Sousa Guerreiro et al., 2007). This is in line with the premise that IBD develops in genetically susceptible hosts but that environmental factors precipitate the onset or reactivation of disease. Determining whether AhR or its downstream elements can play an additional role in determining human disease onset or severity requires future studies.

Our data also make a genetic link between the IELs of the intestine and the skin, both depending on AhR-mediated signals for their maintenance. In mice, adoptive transfer of T lymphocytes

into immunodeficient animals induces both colitis and psoriasis-like phenotypes (Davenport et al., 2002; Leon et al., 2006). A view of an association between IBD and cutaneous disorders (Bernstein et al., 2005; Najarian and Gottlieb, 2003), of which the most frequent example is psoriasis (7%–11% of IBD population versus 1%–2% of general population) (Hoffmann and Kruis, 2004), in line with common susceptibility loci (Cargill et al., 2007; Duerr et al., 2006; Einarsdottir et al., 2009) has been proposed. However, the epidermal TCR$\gamma\delta$ population is absent in human skin, and although an alternative lymphocyte population is present (Duhen et al., 2009), to our knowledge AhR has not been reported as a susceptibility locus to date.

How AhR-mediated signals maintain IELs at epithelial sites is currently unknown and is the subject of further investigation. AhR may regulate particular metabolic pathways within IELs. In line with this, orphan receptors such as AhR have been shown to have strong connections with lipid and hormone metabolic pathways (Fernandez-Salguero et al., 1997; Kliewer et al., 1999), components of which show differential expression in IELs when compared to lymph node-sourced TCR$\alpha\beta^{+}$CD8^{+} T cells (Fahrer et al., 2001). Importantly, metabolic gene expression in IELs is often found to be complementary to that in epithelial cells (Fahrer et al., 2001). In turn, the maintenance of IELs is instrumental in the safeguarding and repair of epithelial-barrier sites, providing protection against damage and microbial invasion, and in controlling the intestinal microfloral load and composition.

In conclusion, our data highlight the evolutionarily highly conserved AhR system as a previously unknown link between external environmental stimuli and the maintenance of specialized immune cell populations (IELs), as well as the control of the microbiota. Our results provide a molecular basis for the importance of cruciferous vegetable-derived phytonutrients as part of a healthy diet in sustaining important elements of the immune system and in controlling bacterial colonization. Furthermore, we reveal an important role for AhR, independent of xenobiotics, in the physiology and homeostasis of epithelial barrier sites.

EXPERIMENTAL PROCEDURES

Mice

C57BL/6 (B6), Rag1-deficient (Rag1$^{-/-}$), Rag1-Cre, floxed AhR (AhR$^{fl/fl}$), and AhR-deficient mice (AhR$^{-/-}$), all on a B6 background, were bred and maintained under specific pathogen-free conditions at NIMR, and all animal experiments were done according to institutional guidelines approved by the local ethical panel and by a project license from the UK Home Office.

T Cell Isolation and FACS Staining

Intestinal IELs were isolated by longitudinal opening of the small intestine. 0.5 cm fragments were shaken for 30 min at 37°C in PBS supplemented with 10% FCS, 10 mM EDTA, 20 mM HEPES, 1 mM sodium pyruvate, and 10 μg/ml Polymyxin B. Single-cell suspensions were further purified using 37.5% isotonic Percoll. Cells were used for analysis or purified using FACS (MoFlo, Beckman Coulter), selecting cells positive for CD8α but negative for TCRβ. After removal of the IEL fraction, the LP fraction was isolated via a digest with 0.4 mg/ml Liberase TL (Roche Applied Science) for a further 60 min at 37°C. Skin IELs were isolated from ears. Skin sheet fragments were shaken for 60 min at 37°C in PBS containing 0.4 mg/ml Liberase TL and 10 μg/ml Collagenase D (Roche Applied Science). Single-cell suspensions were used for analysis or further purification.

For measurements of intracellular cytokines, ex vivo harvested T cells were restimulated with 500 ng/ml phorbol dibutyrate (PdBU), 500 ng/ml ionomycin in the presence of brefeldin A for 90 min. Incorporation of EdU (Invitrogen) was detected according to the manufacturers' instructions.

Real-Time PCR

RNA was extracted using Trizol (Invitrogen) and 1-bromo-3-chloro-propane (Sigma) and reverse-transcribed with oligo d(T)$_{16}$ (Invitrogen Superscript III) according to the manufacturer's protocol. The expression of mRNA for genes of interest in T cell subsets was analyzed after ex vivo isolation using specific primers from Applied Biosystems, and expression was normalized to the housekeeping gene *Hprt*.

Immunofluorescence

Skin samples were stained as described before (Bergstresser and Juarez, 1984). Briefly, ears were split into dorsal and ventral sides and left in Ca^{2+}-, Mg^{2+}-free PBS supplemented with 20 mM EDTA for 2 hr. Epidermal sheets were lifted from the dermis and incubated at −20°C in acetone. Epidermal sheets were washed in PBS and blocked in 2% (w/v) BSA before antibodies were added directly. Sheets were mounted onto silane-coated slides.

Frozen sections of small intestine were prepared at a thickness of 6 μm and fixed in acetone at 4°C. Sections were counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI, Invitrogen) and mounted using DABCO (Sigma-Aldrich). Monoclonal antibodies used were TCR$\gamma\delta$ (eBioGL3, Ebioscience), TCRVγ3 (BD PharMingen), and EpCam (Biolegend). Confocal images were obtained using a LSM 510 Meta microscope (Zeiss) equipped with 405, 488, 543, and 633 nm lasers, and image analysis used the Zeiss LSM software.

EdU staining was performed according to the manufacturer's instructions (Click-iT EdU HCS assay, Invitrogen). Briefly, 1 cm lengths of intestine were fixed in 4% paraformaldehyde, permeabilized in 0.5% Triton X-100, and subsequently embedded in 8% agarose. One hundred nanometer sections were treated with Click-iT reaction cocktails and stained with Hoechst and Azide-Alexa Fluor-647. Confocal images were obtained using a DMIRE2 microscope (Leica) equipped with 405, 488, 543, and 633 nm lasers, and image analysis used Leica software.

DSS Colitis

Dextran sulfate sodium (36,000–50,000 molecular weight, MP Biomedicals) was administered in the drinking water as a 3% (w/v) solution for 6 days, after which it was replaced with normal drinking water. Weights were taken daily, and mice losing 20% of their initial body weight were humanely killed as stipulated in the project license from the UK Home Office governing this work.

Determination of the Intestinal Bacterial Load

DNA from homogenized tissues of identical weight was extracted using the QIAamp DNA Stool kit (QIAgen) according to the manufacturer's protocol for stool pathogen detection. qPCR assays were performed on a CFX96 Real-Time System (BioRad) using Fast SYBR Green Master Mix (Applied Biosystems) and bacteria or phyla-specific primers. For more details, see Extended Experimental Procedures.

Statistical Analysis

For statistical analysis, unpaired Student's t tests were used. Significance of $p < 0.01$is indicated by *. Error bars represent standard deviations unless otherwise specified.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, five figures, and one table and can be found with this article online at doi:10.1016/j.cell.2011.09.025.

ACKNOWLEDGMENTS

We thank A. Rae and G. Preece for assistance with cell sorting; H. Boyes, J. Holland, and T. Norton for assistance with maintenance of mouse strains;

and Drs. B. Stockinger, E. Simpson, G. Butcher, and M. Turner for helpful discussions and advice. The work was supported by the Medical Research Council UK (Ref. U117512792) and the Biotechnology and Biological Sciences Research Council.

Received: March 5, 2011
Revised: August 15, 2011
Accepted: September 16, 2011
Published online: October 13, 2011

REFERENCES

Abreu, M.T. (2010). Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function. Nat. Rev. Immunol. *10*, 131–144.

Amre, D.K., D'Souza, S., Morgan, K., Seidman, G., Lambrette, P., Grimard, G., Israel, D., Mack, D., Ghadirian, P., Deslandres, C., et al. (2007). Imbalances in dietary consumption of fatty acids, vegetables, and fruits are associated with risk for Crohn's disease in children. Am. J. Gastroenterol. *102*, 2016–2025.

Andoh, A., Imaeda, H., Aomatsu, T., Inatomi, O., Bamba, S., Sasaki, M., Saito, Y., Tsujikawa, T., and Fujiyama, Y. (2011). Comparison of the fecal microbiota profiles between ulcerative colitis and Crohn's disease using terminal restriction fragment length polymorphism analysis. J. Gastroenterol. *46*, 479–486.

Ayabe, T., Satchell, D.P., Wilson, C.L., Parks, W.C., Selsted, M.E., and Ouellette, A.J. (2000). Secretion of microbicidal alpha-defensins by intestinal Paneth cells in response to bacteria. Nat. Immunol. *1*, 113–118.

Bergstresser, P.R., and Juarez, D.V. (1984). Detection by immunochemical techniques of cell surface markers on epidermal Langerhans cells. Methods Enzymol. *108*, 683–691.

Bernstein, C.N., Wajda, A., and Blanchard, J.F. (2005). The clustering of other chronic inflammatory diseases in inflammatory bowel disease: a population-based study. Gastroenterology *129*, 827–836.

Bjeldanes, L.F., Kim, J.Y., Grose, K.R., Bartholomew, J.C., and Bradfield, C.A. (1991). Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Proc. Natl. Acad. Sci. USA *88*, 9543–9547.

Bloom, S.M., Bijanki, V.N., Nava, G.M., Sun, L., Malvin, N.P., Donermeyer, D.L., Dunne, W.M., Jr., Allen, P.M., and Stappenbeck, T.S. (2011). Commensal Bacteroides species induce colitis in host-genotype-specific fashion in a mouse model of inflammatory bowel disease. Cell Host Microbe *9*, 390–403.

Boismenu, R., and Havran, W.L. (1994). Modulation of epithelial cell growth by intraepithelial gamma delta T cells. Science *266*, 1253–1255.

Boitano, A.E., Wang, J., Romeo, R., Bouchez, L.C., Parker, A.E., Sutton, S.E., Walker, J.R., Flaveny, C.A., Perdew, G.H., Denison, M.S., et al. (2010). Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. Science *329*, 1345–1348.

Boyden, L.M., Lewis, J.M., Barbee, S.D., Bas, A., Girardi, M., Hayday, A.C., Tigelaar, R.E., and Lifton, R.P. (2008). Skint1, the prototype of a newly identified immunoglobulin superfamily gene cluster, positively selects epidermal gammadelta T cells. Nat. Genet. *40*, 656–662.

Carding, S.R., Kyes, S., Jenkinson, E.J., Kingston, R., Bottomly, K., Owen, J.J., and Hayday, A.C. (1990). Developmentally regulated fetal thymic and extrathymic T-cell receptor gamma delta gene expression. Genes Dev. *4*, 1304–1315.

Cargill, M., Schrodi, S.J., Chang, M., Garcia, V.E., Brandon, R., Callis, K.P., Matsunami, N., Ardlie, K.G., Civello, D., Catanese, J.J., et al. (2007). A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. Am. J. Hum. Genet. *80*, 273–290.

Cong, Y., Brandwein, S.L., McCabe, R.P., Lazenby, A., Birkenmeier, E.H., Sundberg, J.P., and Elson, C.O. (1998). CD4+ T cells reactive to enteric bacterial antigens in spontaneously colitic C3H/HeJBir mice: increased T helper cell type 1 response and ability to transfer disease. J. Exp. Med. *187*, 855–864.

Cua, D.J., and Tato, C.M. (2010). Innate IL-17-producing cells: the sentinels of the immune system. Nat. Rev. Immunol. *10*, 479–489.

D'Souza, S., Levy, E., Mack, D., Israel, D., Lambrette, P., Ghadirian, P., Deslandres, C., Morgan, K., Seidman, E.G., and Amre, D.K. (2008). Dietary patterns and risk for Crohn's disease in children. Inflamm. Bowel Dis. *14*, 367–373.

Davenport, C.M., McAdams, H.A., Kou, J., Mascioli, K., Eichman, C., Healy, L., Peterson, J., Murphy, S., Coppola, D., and Truneh, A. (2002). Inhibition of pro-inflammatory cytokine generation by CTLA4-Ig in the skin and colon of mice adoptively transplanted with CD45RBhi CD4+ T cells correlates with suppression of psoriasis and colitis. Int. Immunopharmacol. *2*, 653–672.

Duerr, R.H., Taylor, K.D., Brant, S.R., Rioux, J.D., Silverberg, M.S., Daly, M.J., Steinhart, A.H., Abraham, C., Regueiro, M., Griffiths, A., et al. (2006). A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. Science *314*, 1461–1463.

Duhen, T., Geiger, R., Jarrossay, D., Lanzavecchia, A., and Sallusto, F. (2009). Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. Nat. Immunol. *10*, 857–863.

Einarsdottir, E., Koskinen, L.L., Dukes, E., Kainu, K., Suomela, S., Lappalainen, M., Ziberna, F., Korponay-Szabo, I.R., Kurppa, K., Kaukinen, K., et al. (2009). IL23R in the Swedish, Finnish, Hungarian and Italian populations: association with IBD and psoriasis, and linkage to celiac disease. BMC Med. Genet. *10*, 8.

Fahrer, A.M., Konigshofer, Y., Kerr, E.M., Ghandour, G., Mack, D.H., Davis, M.M., and Chien, Y.H. (2001). Attributes of gammadelta intraepithelial lymphocytes as suggested by their transcriptional profile. Proc. Natl. Acad. Sci. USA *98*, 10261–10266.

Fernandez-Salguero, P., Pineau, T., Hilbert, D.M., McPhail, T., Lee, S.S., Kimura, S., Nebert, D.W., Rudikoff, S., Ward, J.M., and Gonzalez, F.J. (1995). Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. Science *268*, 722–726.

Fernandez-Salguero, P.M., Ward, J.M., Sundberg, J.P., and Gonzalez, F.J. (1997). Lesions of aryl-hydrocarbon receptor-deficient mice. Vet. Pathol. *34*, 605–614.

Frank, D.N., St Amand, A.L., Feldman, R.A., Boedeker, E.C., Harpaz, N., and Pace, N.R. (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc. Natl. Acad. Sci. USA *104*, 13780–13785.

Fuss, I.J., Neurath, M., Boirivant, M., Klein, J.S., de la Motte, C., Strong, S.A., Fiocchi, C., and Strober, W. (1996). Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. J. Immunol. *157*, 1261–1270.

Garman, R.D., Doherty, P.J., and Raulet, D.H. (1986). Diversity, rearrangement, and expression of murine T cell gamma genes. Cell *45*, 733–742.

Girardi, M., Glusac, E., Filler, R.B., Roberts, S.J., Propperova, I., Lewis, J., Tigelaar, R.E., and Hayday, A.C. (2003). The distinct contributions of murine T cell receptor (TCR)gammadelta+ and TCRalphabeta+ T cells to different stages of chemically induced skin cancer. J. Exp. Med. *198*, 747–755.

Harper, P.H., Lee, E.C., Kettlewell, M.G., Bennett, M.K., and Jewell, D.P. (1985). Role of the faecal stream in the maintenance of Crohn's colitis. Gut *26*, 279–284.

Hoffmann, R.M., and Kruis, W. (2004). Rare extraintestinal manifestations of inflammatory bowel disease. Inflamm. Bowel Dis. *10*, 140–147.

Ismail, A.S., Behrendt, C.L., and Hooper, L.V. (2009). Reciprocal interactions between commensal bacteria and gamma delta intraepithelial lymphocytes during mucosal injury. J. Immunol. *182*, 3047–3054.

Ito, S., Chen, C., Satoh, J., Yim, S., and Gonzalez, F.J. (2007). Dietary phytochemicals regulate whole-body CYP1A1 expression through an arylhydrocarbon receptor nuclear translocator-dependent system in gut. J. Clin. Invest. *117*, 1940–1950.

Kimura, A., Naka, T., Nakahama, T., Chinen, I., Masuda, K., Nohara, K., Fujii-Kuriyama, Y., and Kishimoto, T. (2009). Aryl hydrocarbon receptor in combination with Stat1 regulates LPS-induced inflammatory responses. J. Exp. Med. *206*, 2027–2035.

King, D.P., Hyde, D.M., Jackson, K.A., Novosad, D.M., Ellis, T.N., Putney, L., Stovall, M.Y., Van Winkle, L.S., Beaman, B.L., and Ferrick, D.A. (1999). Cutting edge: protective response to pulmonary injury requires gamma delta T lymphocytes. J. Immunol. *162*, 5033–5036.

Kliewer, S.A., Lehmann, J.M., and Willson, T.M. (1999). Orphan nuclear receptors: shifting endocrinology into reverse. Science *284*, 757–760.

Knutson, J.C., and Poland, A. (1982). Response of murine epidermis to 2,3,7,8-tetrachlorodibenzo-p-dioxin: interaction of the ah and hr loci. Cell *30*, 225–234.

Komano, H., Fujiura, Y., Kawaguchi, M., Matsumoto, S., Hashimoto, Y., Obana, S., Mombaerts, P., Tonegawa, S., Yamamoto, H., Itohara, S., et al. (1995). Homeostatic regulation of intestinal epithelia by intraepithelial gamma delta T cells. Proc. Natl. Acad. Sci. USA *92*, 6147–6151.

Lahvis, G.P., and Bradfield, C.A. (1998). Ahr null alleles: distinctive or different? Biochem. Pharmacol. *56*, 781–787.

Leon, F., Contractor, N., Fuss, I., Marth, T., Lahey, E., Iwaki, S., la Sala, A., Hoffmann, V., Strober, W., and Kelsall, B.L. (2006). Antibodies to complement receptor 3 treat established inflammation in murine models of colitis and a novel model of psoriasiform dermatitis. J. Immunol. *177*, 6974–6982.

Loftus, E.V., Jr. (2004). Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. Gastroenterology *126*, 1504–1517.

Marlowe, J.L., and Puga, A. (2005). Aryl hydrocarbon receptor, cell cycle regulation, toxicity, and tumorigenesis. J. Cell. Biochem. *96*, 1174–1184.

Martin, B., Hirota, K., Cua, D.J., Stockinger, B., and Veldhoen, M. (2009). Interleukin-17-producing gammadelta T cells selectively expand in response to pathogen products and environmental signals. Immunity *31*, 321–330.

Maslowski, K.M., Vieira, A.T., Ng, A., Kranich, J., Sierro, F., Yu, D., Schilter, H.C., Rolph, M.S., Mackay, F., Artis, D., et al. (2009). Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature *461*, 1282–1286.

Mazmanian, S.K., Round, J.L., and Kasper, D.L. (2008). A microbial symbiosis factor prevents intestinal inflammatory disease. Nature *453*, 620–625.

Mombaerts, P., Arnoldi, J., Russ, F., Tonegawa, S., and Kaufmann, S.H. (1993). Different roles of alpha beta and gamma delta T cells in immunity against an intracellular bacterial pathogen. Nature *365*, 53–56.

Moore, T.A., Moore, B.B., Newstead, M.W., and Standiford, T.J. (2000). Gamma delta-T cells are critical for survival and early proinflammatory cytokine gene expression during murine Klebsiella pneumonia. J. Immunol. *165*, 2643–2650.

Najarian, D.J., and Gottlieb, A.B. (2003). Connections between psoriasis and Crohn's disease. J. Am. Acad. Dermatol. *48*, 805–821, quiz 822–824.

Oberg, M., Bergander, L., Håkansson, H., Rannug, U., and Rannug, A. (2005). Identification of the tryptophan photoproduct 6-formylindolo[3,2-b]carbazole, in cell culture medium, as a factor that controls the background aryl hydrocarbon receptor activity. Toxicol. Sci. *85*, 935–943.

Pellequer, J.L., Wager-Smith, K.A., Kay, S.A., and Getzoff, E.D. (1998). Photoactive yellow protein: a structural prototype for the three-dimensional fold of the PAS domain superfamily. Proc. Natl. Acad. Sci. USA *95*, 5884–5890.

Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S., and Medzhitov, R. (2004). Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell *118*, 229–241.

Roberts, S.J., Smith, A.L., West, A.B., Wen, L., Findly, R.C., Owen, M.J., and Hayday, A.C. (1996). T-cell alpha beta + and gamma delta + deficient mice display abnormal but distinct phenotypes toward a natural, widespread infection of the intestinal epithelium. Proc. Natl. Acad. Sci. USA *93*, 11774–11779.

Rocha, B., Vassalli, P., and Guy-Grand, D. (1994). Thymic and extrathymic origins of gut intraepithelial lymphocyte populations in mice. J. Exp. Med. *180*, 681–686.

Sadek, C.M., and Allen-Hoffmann, B.L. (1994). Cytochrome P450IA1 is rapidly induced in normal human keratinocytes in the absence of xenobiotics. J. Biol. Chem. *269*, 16067–16074.

Schmidt, J.V., Su, G.H., Reddy, J.K., Simon, M.C., and Bradfield, C.A. (1996). Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. Proc. Natl. Acad. Sci. USA *93*, 6731–6736.

Selin, L.K., Santolucito, P.A., Pinto, A.K., Szomolanyi-Tsuda, E., and Welsh, R.M. (2001). Innate immunity to viruses: control of vaccinia virus infection by gamma delta T cells. J. Immunol. *166*, 6784–6794.

Shanahan, F. (2000). Immunology. Therapeutic manipulation of gut flora. Science *289*, 1311–1312.

Sokol, H., Seksik, P., Rigottier-Gois, L., Lay, C., Lepage, P., Podglajen, I., Marteau, P., and Doré, J. (2006). Specificities of the fecal microbiota in inflammatory bowel disease. Inflamm. Bowel Dis. *12*, 106–111.

Sousa Guerreiro, C., Cravo, M., Costa, A.R., Miranda, A., Tavares, L., Moura-Santos, P., MarquesVidal, P., and Nobre Leitão, C. (2007). A comprehensive approach to evaluate nutritional status in Crohn's patients in the era of biologic therapy: a case-control study. Am. J. Gastroenterol. *102*, 2551–2556.

Taylor, B.L., and Zhulin, I.B. (1999). PAS domains: internal sensors of oxygen, redox potential, and light. Microbiol. Mol. Biol. Rev. *63*, 479–506.

Veldhoen, M., Hirota, K., Westendorf, A.M., Buer, J., Dumoutier, L., Renauld, J.C., and Stockinger, B. (2008). The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature *453*, 106–109.

Veldhoen, M., Hirota, K., Christensen, J., O'Garra, A., and Stockinger, B. (2009). Natural agonists for aryl hydrocarbon receptor in culture medium are essential for optimal differentiation of Th17 T cells. J. Exp. Med. *206*, 43–49.

Vijay-Kumar, M., Sanders, C.J., Taylor, R.T., Kumar, A., Aitken, J.D., Sitaraman, S.V., Neish, A.S., Uematsu, S., Akira, S., Williams, I.R., and Gewirtz, A.T. (2007). Deletion of TLR5 results in spontaneous colitis in mice. J. Clin. Invest. *117*, 3909–3921.

Walisser, J.A., Glover, E., Pande, K., Liss, A.L., and Bradfield, C.A. (2005). Aryl hydrocarbon receptor-dependent liver development and hepatotoxicity are mediated by different cell types. Proc. Natl. Acad. Sci. USA *102*, 17858–17863.

Wehkamp, J., Salzman, N.H., Porter, E., Nuding, S., Weichenthal, M., Petras, R.E., Shen, B., Schaeffeler, E., Schwab, M., Linzmeier, R., et al. (2005). Reduced Paneth cell alpha-defensins in ileal Crohn's disease. Proc. Natl. Acad. Sci. USA *102*, 18129–18134.

Wilson, C.L., Ouellette, A.J., Satchell, D.P., Ayabe, T., López-Boado, Y.S., Stratman, J.L., Hultgren, S.J., Matrisian, L.M., and Parks, W.C. (1999). Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. Science *286*, 113–117.

Wirtz, S., Neufert, C., Weigmann, B., and Neurath, M.F. (2007). Chemically induced mouse models of intestinal inflammation. Nat. Protoc. *2*, 541–546.

Xiong, N., Kang, C., and Raulet, D.H. (2002). Redundant and unique roles of two enhancer elements in the TCRgamma locus in gene regulation and gammadelta T cell development. Immunity *16*, 453–463.