nature immunology

Epithelial decision makers: in search of the 'epimmunome'

Mahima Swamy[1,2], Colin Jamora[3], Wendy Havran[4] & Adrian Hayday[1,2]

Frequent microbial and nonmicrobial challenges to epithelial cells trigger discrete pathways, promoting molecular changes such as the secretion of specific cytokines and chemokines and alterations to molecules displayed at the epithelial cell surface. In combination, these molecules impose key decisions on innate and adaptive immune cells. Depending on context, those decisions can be as diverse as those imposed by professional antigen-presenting cells, benefiting the host by balancing immune competence with the avoidance of immunopathology. Nonetheless, this potency of epithelial cells is also consistent with the causal contribution of epithelial dysregulation to myriad inflammatory diseases. This pathogenic axis provides an attractive target for tissue-specific clinical manipulation. In this context, a research goal should be to identify all molecules used by epithelial cells to instruct immune cells. We term this the 'epimmunome'.

Epithelial cells in the thymus can initiate a range of responses from progenitor thymocytes by providing tissue-restricted antigens for the T cell antigen receptor (TCR); costimulatory functions, such as Notch ligands; and cytokines, such as interleukin (IL)-7 (ref. 1). In the periphery, however, the capacity of epithelial cells to initiate immune responses has been undervalued, in part because of the anatomical separation of epithelial cells from naive T cells. Instead, the role of initiating lymphocyte responses has been passed to dendritic cells (DCs), which can carry molecular information from tissues to naive T cells in the lymph nodes. Indeed, for many years the primary contribution of body surface epithelia to host protection was viewed as physicochemical effector functions. In cases of epithelial barrier disruption or pathogen invasion, a somewhat unconnected, systemic immune response would be invoked by direct microbial challenge.

This assessment is being substantively revised, largely on the basis of two strands of evidence. First, it is increasingly clear that the direct response of epithelial cells to infection and/or stress can strongly influence DCs and their subsequent regulation of adaptive responses. Second, there is emerging evidence for the direct activation by epithelial cells of lymphoid and myeloid cell repertoires that are constitutively tissue associated. This re-evaluation coincides with growing evidence from mouse models and from human genetics that epithelial cell dysregulation in different tissues can be a primary cause of inflammatory pathology. Such evidence identifies the clinical potential of targeting body surface–specific inflammatory pathways, which may prove preferable to the long-term blockade of key systemic pathways.

[1]London Research Institute, Cancer Research UK, London, UK. [2]Peter Gorer Department of Immunobiology, King's College London at Guy's Hospital, London, UK. [3]Section of Cell and Developmental Biology, Division of Biological Sciences, University of California San Diego, La Jolla, California, USA. [4]Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, California, USA. Correspondence should be addressed to W.H. (havran@scripps.edu) or A.H. (adrian.hayday@kcl.ac.uk).

Published online 20 July 2010; doi:10.1038/ni.1905

Anatomical epithelial-immune integration

Epithelial cells are the main constituent of tissues lining the surfaces of organs or internal cavities. Consequently, they are involved in a plethora of processes, including the intricate regulation of secretion and absorption in organs such as the skin, gut and lungs, and the protection of the subepithelial compartments from the pathogenic microorganisms, toxic factors and physical trauma to which they are directly exposed. The essential building blocks of the tissues are the epithelial cell sheets, which are constructed in large part by various forms of intercellular adhesive junctions. These adhesion apparatuses determine the shape and polarity of the epithelial cell and the complexity of the tissue that will form, be it simple or stratified.

In several tissues, intercellular adhesion molecules also mediate the physical integration of epithelial cells with resident immune cells. In murine skin, for instance, Langerhans cells and dendritic intraepidermal γδ T cells (DETCs) infiltrate the epidermis during the stratification of keratinocytes. Such epithelial-immune integration is to be distinguished from the infiltration of epithelia by systemic immune cells in response to inflammation, and it seems increasingly likely that Langerhans cells and DETCs both represent tissue-restricted, self-renewing immune compartments. Intraepithelial lymphocytes (IELs) are likewise found in many other organs, most notably the intestine. They comprise primarily a variable mixture of conventional, $CD8^+$ αβ T cells that recognize peptides presented by major histocompatibility class I (MHC-I) antigens, and unconventional, non-MHC-restricted αβ or γδ T cells that express neither the CD4 nor CD8 αβ coreceptors[2]. IEL compartments are often much less diverse than their systemic counterparts, but, given the surface area of epithelia, they can collectively compose a major fraction of the body's T cells. They are rapidly responsive, as if in an 'activated yet resting' state[2], and have the capacity to eradicate dysregulated epithelial cells by cytolysis. Nonetheless, they are functionally pleiotropic, with aggregate immunoregulatory as well as immunoprotective functions[3].

Epithelial cells can directly support IEL development. For example, crossing an intestinal epithelial cell–specific *Il7* transgene into an *Il7*$^{-/-}$ mouse was sufficient to rescue the development of intestinal γδ IELs that are IL-7 dependent[4]. Likewise, epithelial cell–derived IL-15 is a survival factor for resting IELs and a proliferative factor for TCR-activated IELs[5]. Although not all epithelia constitutively harbor IELs, other specialized, tissue-associated lymphocyte subsets may periodically enter the epithelium from the immediate subepithelial space. Such may be the case for human cutaneous T cells that produce IL-22 and several fibroblast growth factors[6]. Indeed, the multiplicity of immune cell types associated with body surfaces is increasingly apparent. To cite but two recent examples, the gut constitutively harbors an natural killer (NK)-like cell type that also produces IL-22 (ref. 7), and an ill-defined innate immune cell that produces IL-17 and interferon (IFN)-γ in response to IL-23 (ref. 8). Thus, the developmental integration of epithelial, myeloid and lymphoid cells within tissues of defined structure and polarity constructs a local immune surveillance system, in which epithelial cells may function as primary sentinels.

Epithelial cell dysregulation initiates inflammation

Alterations in epithelial sheet structure caused by wounding, abrasion or dysfunction of the cornification program can elicit myriad changes, including the rapid proliferation of epithelial progenitor cells. This itself can be immunoregulatory. For example, proteins upregulated in the hyperplastic environment include the calcium-binding protein calprotectin, a nuclear heterodimer of S100A8 and S100A9 that can affect the epithelial cell's differentiation program[9]. However, S100 proteins may also be secreted and function as chemoattractants[10] and activators of myeloid cells or lymphocytes by binding to the immunoglobulin superfamily member RAGE and/or by enhancing the effects of lipopolysaccharide (LPS) on Toll-like receptor (TLR)-4 (ref. 11). Engagement of RAGE or TLR4 activates myeloid cells and can also delay activation-induced apoptosis, thereby promoting inflammation. Thus, the physiologic status of the epithelial barrier determines immune cell activity.

The relevance of the S100-RAGE pathway is implied by the capacity of antibodies to S100 to reduce inflammation in several models[10]. Interestingly, S100A8 and S100A9 expression is induced by IL-1α, which is likewise expressed by keratinocytes[12] (see below). Germane to this, transgenic mice overexpressing β1 integrin in keratinocytes show high levels of IL-1α, keratinocyte hyperproliferation and spontaneous skin inflammation[13] that may be partly attributable to S100 activity. S100 biology is reminiscent of the reported double life of high mobility group binding protein-1 (HMGB1), which regulates chromatin but, upon release from dying epithelial cells, may also engage RAGE and/or TLR4, for example on neighboring DCs[14]. RAGE activation may be further influenced by local oxidative, hyperglycemic and hypoxic stress. The remodeling of surface sugar moieties is another example of an epithelial cell response to barrier dysregulation[15]. For instance, at the onset of epithelial trauma, heparan sulfate proteoglycans are upregulated[16]. One such keratinocyte-specific heparan sulfate proteoglycan, the V3 splice variant form of CD44, seems to mediate close interactions of keratinocytes with skin-infiltrating T cells in the development of cutaneous inflammation[17].

Inflammation initiated by misfolded epithelial proteins

The unfolded protein response (UPR) is a multifaceted reaction of cells to problems in protein handling that follow from infection, hyperproliferation and physico-chemical and metabolic dysregulation. Under conditions of endoplasmic reticulum (ER) stress, the UPR attempts to resolve the misfolding problem by downregulating translation, degrading misfolded proteins and selectively synthesizing more protein-folding chaperones. The UPR may in turn activate autophagy, but if this and other measures fail, it will invoke programmed cell death. In addition to these cell-autonomous effects, the UPR mediates interactions with immune cells[18]. Secretory intestinal epithelial cells (IECs), such as Paneth and goblet cells, are particularly prone to invoke the UPR.

The UPR is largely mediated by three ER transmembrane proteins, including the inositol-requiring kinase/endonuclease 1 (IRE1). This highly conserved protein comprises a dimer of ubiquitously expressed α-subunits or includes an intestinal epithelial cell–specific β-subunit[19]. When activated from a latent state, IRE1 cleaves 26bp from unspliced X-box-binding protein 1 (*XBP1*) mRNA to yield *XBP1s*, whose product transcriptionally activates major portions of the UPR. When a mutation that blocks conversion to *XBP1s* was introduced specifically into IECs, the resulting mice spontaneously developed neutrophilic infiltration that variably progressed to full colitis[20]. Notably, the mice overexpressed the neutrophilic chemokine CXCL1 and other effectors in response to tumor necrosis factor (TNF) and to bacterial flagellin, two agents commonly implicated in inflammation. These data establish the capacity of the epithelial UPR to regulate neutrophils and other immune cells, identifying it as a fundamental fulcrum for whether an organ tips into inflammatory pathology. The relevance of this to human disease is evident in the genetic association of *XBP1* variants with ulcerative colitis and Crohn's disease, the two main forms of inflammatory bowel disease (IBD)[18]. Likewise, the genetic association of IBD with loci encoding autophagy and inflammasome regulators, such as ATG16L1 (ref. 21), seemingly reflects the importance of appropriate epithelial regulation of these related pathways (see below).

Inflammation may be promoted by exaggerated as well as defective UPR. Mucin-2, a major component of the luminal glycocalyx secreted by intestinal goblet cells, is a large, heavily glycosylated protein whose assembly and sorting impose pressure on the cell's synthetic machinery. Notably, an ethylnitrosourea mutagenesis screen for colitis identified two mouse strains (Winnie and Eeyore) with mucin-2 dysregulation in goblet cells[22]. Each mutation is associated with high ER stress; high intestinal IL-1β, TNF, IL-13 and IFN-γ; and high propensity to inflammatory disease. Moreover, the likely significance of the UPR to intestinal inflammation is strongly suggested by its regulation by the immunosuppressive cytokine IL-10. *Il10*$^{-/-}$ mice spontaneously develop colitis[23], and mutations in either chain of the human IL-10 receptor show causal association with IBD[24]. When added to the mouse enterocyte line MODE-K, IL-10 suppresses key components of the UPR, suggesting that its anti-inflammatory role in the gut may in part be attributable to its actions on epithelial cells[25].

The evidence that epithelial cells can initiate immune cell responses raises some fundamental questions. For example, what are the main epithelial cell pathways implicated in the regulation of immunity, and what are the key afferent stimuli that feed into those pathways? What molecules do epithelial cells display on their surface or secrete so as to communicate the state of the epithelium to the immune compartment? What types of immune cells do epithelial cells influence? Finally, how diverse are the aggregate immune responses that result from epithelial-immune interactions? This Review will address these issues.

The central role of epithelial NF-κB

Several experiments have established a central role for the epithelial cell NF-κB pathway in regulating immune cell biology. NF-κB is activated

downstream of many pattern recognition receptors (PRRs) and pro-inflammatory cytokines through the activation of the IκB kinase (IKK) complex. The IKK complex consists of the regulatory subunit IKKγ (also known as NEMO) and the effector kinase subunits IKKα and IKKβ, which phosphorylate IκB, thus releasing NF-κB from inhibition. IKKγ$^{IEC\text{-}KO}$ mice, bearing IEC-specific deletion of *Ikbkg*, show defects in barrier function, increased susceptibility of IECs to TNF-induced apoptosis, increased translocation of bacteria across the gut wall and spontaneous TNF receptor–dependent colitis[26]. The production of TNF amplifies the pathology by causing further IEC apoptosis and barrier disruption. This general route to chronic inflammation is not restricted to the intestine, as hepatocyte-specific deletion of *Ikbkg* promotes spontaneous steatohepatitis, TNF-mediated liver damage, fibrosis and the development of hepatocellular carcinoma[27]. Returning to the gut, IKKγ$^{IEC\text{-}KO}$ mice crossed to mice lacking MyD88, the principal adaptor downstream of TLRs and of the IL-1 receptor, show no inflammation[26]. Thus, TLR-mediated recognition of microbes and/or IL-1 can be a critical cofactor for the induction of inflammatory disease. This perspective is supported by genetic data that polymorphisms in bacteria-sensing receptors are causative in IBD (see below).

Mutations restricted to skin epithelial cells can likewise initiate inflammatory disease. When *Ikbkb*, encoding the IKKβ subunit, is specifically deleted in keratinocytes, the newborn mice seem essentially normal[28]. However, the skin rapidly transforms, showing epidermal thickening and focal parakeratosis (common symptoms of psoriasis), myeloid and lymphoid cell infiltration of the dermis and substantially more cutaneous IL-1. Intercrossing the mice to TNF receptor–deficient mice abrogates the disease phenotype. This demonstrates that cell-autonomous keratinocyte defects resulting from NF-κB dysfunction (which would still be present in the intercrossed mice) are not sufficient to provoke inflammation. Moreover, because they lack IKKβ, the keratinocytes cannot themselves respond to TNF, implying that the inflammatory pathology is driven by the responses to TNF of nonepithelial cells (for example, immune cells and/or mesenchymal cells). Collectively, these data argue that episodic encounter of environmental microbial and/or nonmicrobial ligands by afferent receptors on epithelial cells triggers the NF-κB pathway. NF-κB dictates the expression of gene products whose aggregate effects are to maintain immune cells in an anti-inflammatory mode. These products may include antimicrobial defensins that limit microbial invasion and immunoregulatory cytokines (see below). When this level of regulation does not operate, as in the case of IKKβ disruption, nonepithelial cells are induced to produce TNF that acts on immune cells to amplify the inflammatory response. The fact that removing the TNF response completely ameliorates pathology in the transgenic mice despite ongoing dysfunction in the epithelial cells evokes the clinical efficacy of TNF blockade in many multigenic inflammatory diseases.

Intestinal epithelial cells skew immunity

A prototypic, immunostimulatory, epithelial NF-κB pathway was exposed by experiments in which villin expression control elements were used to disrupt IKKβ in enterocytes[29]. No obvious spontaneous disease develops in these IKKβ$^{IEC\text{-}KO}$ mice. However, an afferent stimulus of infection by the intestinal nematode *Trichuris muris* provokes chronic intestinal inflammation attributable to the failure of *Ikbkb*$^{-/-}$ enterocytes to produce an IL-7-like cytokine, thymic stromal lymphopoietin (TSLP). TSLP is highly pleiotropic[30]. It instructs neighboring CD11c^{+} DCs to upregulate OX40L, the antiparasitic effector RELMβ, and other factors that promote protective type-2 immune responses, characterized by high levels of IL-4, IL-5 and IL-13. Moreover, it augments type-2 cytokine production by direct effects on CD4^{+} αβ T cells and by indirect effects on mast cells, natural killer T cells and basophils (see also below). Thus, IKKβ$^{IEC\text{-}KO}$ mice fail to control parasite growth and instead show strong elevation of IL-12, IL-23, IFN-γ and IL-17, which promote rapid progression to severe intestinal inflammation[29].

This study argues that epithelial cell activation can be the critical adjuvant in rendering a challenge immunogenic, and it shows that an epithelial product can skew the phenotype of the resulting adaptive response. Both these properties are conventionally assigned to DCs. However, some important issues remain unresolved. For example, although the IFN-γ and IL-17 immune responses are provoked by infection, and although the IKKβ–NF-κB pathway is downstream of microbe-sensing PRRs, the pathway is, as already mentioned, activated by IL-1 family cytokines and by other forms of cellular stress. Therefore, the production of TSLP and related molecules may reflect the epithelial cells' direct recognition of microbes, a response to IL-1, and/or a stress response to cellular dysregulation imposed by infection.

Germane to this are data provided by a new study of the role of TSLP in inducing cutaneous type-2 immune responses to papain, which is a cysteine protease and an allergen[31]. Although the study focused on the importance of DCs in the initiation of the immune response, it is noteworthy that subcutaneous immunization with nominal antigen and papain provokes substantial production of reactive oxygen species (ROS) by epidermal cells. This is linked to upregulation of TSLP by keratinocytes; IL-12 downregulation by DCs; and basophil recruitment to the draining lymph nodes, all of which promote type-2 T cell differentiation. Furthermore, TSLP induction depends on TLR4 (which can be engaged both by microbial moieties and by oxidized endogenous lipoproteins) and its downstream signaling adaptor TRIF (Toll–IL-1 receptor domain–containing adaptor inducing interferon-β). Collectively, these data suggest that epithelial, NF-κB-mediated, TLR-dependent TSLP upregulation conspires with an oxidative stress response and the engagement of protease-activated receptors (for example, PAR2) to drive adaptive type-2 immune responses to papain and related allergens. The consequent effects on DCs and basophils may then critically amplify this skew toward type-2 immunity, rather than initiating it *de novo*. Indeed, the upregulation of TSLP downstream of PAR2 implies that many allergen-associated proteases may initiate type-2 responses by promoting epithelial cell expression of TSLP[32].

Afferent stimuli for epithelial cells

In establishing the respective contributions of microbial and nonmicrobial stress to epithelial–immune cell interactions, it is noteworthy that the bulk of research on microbial sensing has focused on DCs, monocytes and macrophages, rather than on epithelial cells. This is inappropriate. The normal development of gut-associated lymphoid tissue requires microbes, and it is possible that the initial microbe-sensing events that determine this are mediated by epithelial PRRs. However, we remain uncertain which epithelial cell subsets, in which locations and at which point in differentiation express specific members of the seven best known PRR classes—TLRs, NOD-like receptors (NLRs), pentraxins, mannose-binding lectins, RIG-I and the dectin and HIN200 families.

So far, it seems that different epithelial cell subsets express different combinations of cell-surface and endosomal TLRs[33]. Indirect evidence that IEC TLR signaling regulates microbial penetration derives from the high abundance of commensal bacteria found in the spleens of mice lacking the two adaptors, MyD88 and TRIF, that

jointly capture all TLR signaling[34]. Moreover, mice with intestinal epithelium–specific suppression of the MyD88 pathway develop an age-dependent inflammation that may reflect chronic stimulation of systemic myeloid and lymphoid cells by microbes that have penetrated the epithelium[35]. Indeed, use of wild-type bone marrow chimeras to selectively reconstitute microbe-sensing competence in different cellular compartments of *Myd88*$^{-/-}$ mice has attributed several functions to nonhematopoietic cells that include epithelial cells. These range from immunoprotective production of antimicrobial effectors to immunosuppressive and immunoregulatory effects of discrete cytokines[36].

Nod1, a PRR recognizing diaminopimelic acid, is highly expressed in epithelial cells, as well as DCs, T cells and B cells. *Nod1*$^{-/-}$ mice are highly susceptible to infection (for example, by *H. pylori*), being generally impaired in adaptive T cell responses[37]. Notably, those impairments are not fully rescued by reconstituting wild-type lymphoid and myeloid compartments. Specifically, peritoneal administration of Nod1 ligands to such mice fails to induce CXCL1, CCL2 and other inflammatory cytokines and chemokines. As a result, type-2 T cell responses are severely impaired, demonstrating the role *in vivo* of nonhematopoietic cells in orchestrating type-2 adaptive immune responses downstream of Nod1 activation.

Strong genetic associations of Crohn's disease have emerged for IL-23, IL-23R and HLA; for NALP3 (see below); and for Nod2, a PRR sensing the bacterial product muramyl dipeptide[21]. Besides macrophages, primary IECs and many human epithelial cell lines show TNF- and IFN-γ-responsive Nod2 expression. Intestinal Paneth cells of *Nod2*$^{-/-}$ mice secrete smaller amounts of antimicrobial defensins in response to bacterial infections, and these mice are more susceptible to oral infection by *Listeria monocytogenes*[38]. Notably, the increased susceptibility to infection is restricted to the oral route. This, together with evidence that *Nod2*$^{-/-}$ macrophages can still respond to and kill *L. monocytogenes*, strongly implies that the phenotype of *Nod2*$^{-/-}$ mice is attributable to defective epithelial Nod2 sensing. As with *Nod1*$^{-/-}$ mice, chemokine and cytokine defects in *Nod2*$^{-/-}$ mice argue that Nod2 expressed by nonhematopoietic cells promotes type-2 T cell responses in the context of muramyl dipeptide and antigen[39].

Recently, epithelial cells were also shown to form autophagosomes in response to Nod1 and Nod2 ligands[40]. In particular, MODE-K cells form autophagosomes around intracellular *Shigella flexneri* bacteria, with Nod2 and ATG16L1 both recruited to the site of entry. Although fibroblasts are also capable of this, the exposure of IECs to luminal bacteria suggests that autophagosome formation may be critical to the cells' capacity to limit bacterial penetration and thereby to limit immune cell activation. Furthermore, a capacity of epithelial cells to digest bacteria may permit them to process antigens and act *in situ* as antigen-presenting cells (APCs) (see below).

In sum, afferent microbe sensing by epithelial cells occurs by means of several pathways, each of which may influence the cells' regulation of the immune system. Indeed, it is most likely the combinatorial activation of afferent microbial and/or nonmicrobial receptors that ultimately determines the outcome for the immune system—for example, whether specific TLR or Nod activation events occur in tandem and with or without coincident stress signals, such as the perturbation of integrins considered at the beginning of this Review or the physico-chemical challenges to be considered below. The different outcomes of epithelial cell activation will include the selective regulation of different tissue-associated immune cell subtypes. For example, tissue-associated T cells, NK-like cells, and CD11c^{+} DC can each produce IL-22 (refs. 6,7,41). This seeming redundancy may ensure that IL-22 will be made in response to different patterns of epithelial cell activation that respectively activate some but not all of the IL-22-producing cell types. The same reasoning may explain the multiplicity of cell types that express IL-18, IL-6 and fibroblast growth factors (see below). The temporal ordering of events is another factor that will dictate the outcome of epithelial cell activation. Thus, aggregate proinflammatory effects of NF-κB signaling in the acute phase give way to NF-κB-dependent immunosuppressive and tissue-repair responses during the subsequent resolution of inflammation[42]. Therefore, future research should describe the spatial and temporal complexity of the afferent stimuli that determine different epithelial cell functions, which in turn instruct distinct immune responses.

Diverse epithelial effectors

By channeling different afferent stimuli through key signaling pathways, epithelial cells can regulate communication with different sets of immune cells by the selective production of cytokines and chemokines. This goes far beyond the 'default position', wherein immune cell activation is directly caused by microbes that have traversed a damaged epithelium. Indeed, it is attractive to posit pathways by which different states of the epithelium are sensed by different sets of afferent stimuli that activate different combinations of cytokines, thereby dictating an appropriate type of immune response: for example, IFN-γ and/or IL-17A and IL-17F responses to microbial penetration; IL-4, IL-5, IL-13 and/or immunosuppressive responses to abraded but not disrupted epithelia; cytolytic responses to virus-infected or transformed epithelial cells; and wound-healing responses to barrier disruption. Thus, much research activity aims to define such pathways.

TSLP, IL-25 and IL-33

We have already reviewed scenarios in which epithelial cell TSLP induces type-2 T cell responses. IL-25 (IL-17E) is likewise produced by epithelial cells and is also required for the immunoprotective type-2 response of mice to *T. muris*[43]. The main effects of IL-25 require an adaptive immune response, consistent with its effects on CD4^{+} T cells[44]. However, IL-25 in also an autocrine factor, promoting epithelial cell expression of TSLP[45]. As well as contributing to host protection, TSLP and IL-25 contribute to type-2 inflammatory diseases (**Fig. 1**). Thus, mice transgenic for TSLP in airway epithelial cells develop progressive pulmonary inflammation closely resembling human asthma[46]. Patients with atopic dermatitis show high cutaneous TSLP concentrations[47], and mice with an inducible, epidermis-specific TSLP transgene develop an atopic dermatitis–like pathology[48]. Large amounts of *Il25* mRNA have also been identified in intraepithelial lymphocytes[49], formally qualifying conclusions about the main source of this cytokine in different situations. Nonetheless, the production of IL-25 by IELs may itself be a downstream effect of epithelial activation (see below).

IL-33, another cytokine produced mainly by epithelial cells, binds the surface receptor ST2 on type-2 cytokine–producing CD4^{+} T cells[50], increasing their functional differentiation, while also increasing the survival of mast cells and basophils and their production of type-2 cytokines. IL-33 is highly expressed by epithelial cells in ulcerative colitis, the type-2 form of IBD[51] (**Fig. 1**). The formation and secretion of biologically active IL-33 seems to be tightly regulated, as is the case for its cousins in the IL-1 family. Indeed, it has been postulated that intracellular stores of IL-33 function as an 'alarmin' when released in large quantities upon apoptosis or necrosis[52].

As well as their promotion of type-2 adaptive immunity, TSLP and IL-25 can be immunosuppressive. For example, epithelial NF-κB-mediated sensing of commensal bacteria upregulates IL-25 (ref. 53), which selectively suppresses IL-23 production by intestinal DCs.

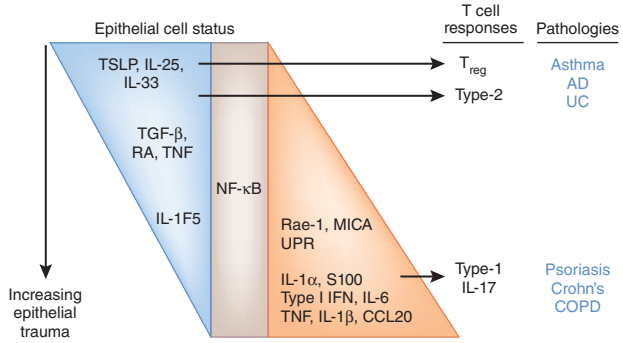

Figure 1 Epithelial cells impose diverse decisions on immune cells. Epithelial cells under various conditions express molecular mediators of immune cell instruction. When stress to the epithelium is relatively mild—for example, abrasion but no overt penetration (top/blue)—the dominance of TSLP, IL-25, IL-33 and other molecules shown instructs T-regulatory and type-2 responses that, when dysregulated, are associated with diseases such as asthma, atopic dermatitis (AD) and ulcerative colitis (UC). As epithelial damage and dysregulation increases (bottom/orange)—for example, as caused by invasive pathogens—other molecules shift the instructions to T cells toward type-1 responses, with a different set of associated diseases. The NF-κB pathway is used by epithelial cells to mount both sets of responses; likewise, molecules in the middle of the figure may contribute to different functional responses. RA, retinoic acid; COPD, chronic obstructive pulmonary disease.

NF-κB signaling further strengthens this immunosuppressive pathway by maintaining expression of IL-17RB, the receptor for IL-25 (ref. 54). Likewise, TSLP inhibits IL-12 expression by intestinal DCs, simultaneously promoting their activation of Foxp3$^+$ regulatory T (T_{reg}) cells[55]. TSLP is reportedly expressed at lower levels by colonic IECs in Crohn's disease (the type-1 form of IBD)[56], whereas it is upregulated by vitamin D3 mimetics that promote T_{reg} activity[57]. A subset of human intestinal, CD103$^+$ DCs seemingly adopts a tolerogenic, T_{reg}-inducing phenotype in response to retinoic acid, transforming growth factor-β (TGF-β) and TSLP, all produced by IECs[58]. Indeed, retinoic acid and TGF-β contribute substantially to tolerogenic responses to noninvasive antigens encountered at body surfaces[59].

Naturally, were IECs to induce an unresponsive state in all gut DC subsets, it would be impossible to trigger adaptive immunity to pathogens. Again, the solution lies, at least in part, with the epithelial cell that, in response to TLR ligation, secretes the chemokine CCL20, which recruits immature DCs that have not been pretolerized[60]. Additionally, MyD88-dependent signaling induces IECs to release CX3CL1 (fractalkine), which in some mouse strains promotes DC dendrite extension into the gut lumen, permitting direct microbial activation[61]. Nonetheless, the threshold for the shift in adaptive immunity may be high, confounding a common perception that type-2 responses are somehow less robust than type-1 responses. Thus, type-2 immune responses elicited by the concerted actions of three epithelial cytokines may impose dominance over existing or newly primed type-1 responses. This realization offers new clinical approaches for suppressing type-1 and/or IL-17-rich autoimmune responses. It may also explain the persistence of allergic responses in the context of ongoing type-1 responses[62].

The IL-1 family

Conspicuous among epithelial cytokines are IL-1 family members, which bear strong evolutionary relationships to fibroblast growth factors (FGFs). IL-1α (IL-1F1) can be constitutively and inducibly expressed by epithelial cells in different tissues[63]. It has autocrine activity, among other things promoting production of antimicrobial peptides that limit microbial penetration and hence the level of subepithelial immune cell activation. It also has paracrine activity; thus, IL-1α produced by prostatic epithelial cells provokes local stromal cells to produce insulin-like growth factor (IGF)-1, which in turn promotes the growth of the epithelial cells[64]. Interestingly, this developmental role of IL-1α, which is attested to by impaired prostatic development in IL-1 receptor–deficient mice, is recapitulated in inflammatory prostatitis. However, in this case, IL-1α is also produced by infiltrating immune cells, and substantial amounts of IL-1β and IL-18 are also produced that do not contribute to the development of the prostate[64]. During inflammation, IL-1α can drive macrophage and T cell activation. Thus, transgenic mice with IL-1α expression specifically in keratinocytes show dermal macrophage infiltration and focal inflammatory lesions, although without the epidermal thickening and increased proliferation associated with psoriasis[65].

As mentioned, both IL-1β (IL-1F2) and IL-18 (another IL-1 family member) can be inducibly produced by epithelial cells[66]. Their processing requires caspase-mediated cleavage, and both cytokines have been intensively studied as the primary products of the NALP3-dependent, inflammasome-mediated stress response[67]. Recent studies have identified stress-dependent NALP3 induction in keratinocytes[68] and in IECs[69,70]. In the latter case, intestinal stress imposed by sodium dextran sulfate (DSS) provokes IL-18 but not IL-1β production by nonhematopoietic cells that are most likely IECs. Mice lacking the inflammasome-associated caspase 1 show severe susceptibility to DSS-induced colitis that can be ameliorated by provision of IL-18, which provokes epithelial cell proliferation and wound healing. Consistent with this, *Il18*$^{-/-}$ mice also show high susceptibility to DSS-induced colitis and have wound-healing defects[71].

IL-1F6, IL-1F8 and IL-1F9 are largely epithelial, and transgenic mice with IL-1F6 expressed specifically by keratinocytes develop widespread cutaneous inflammation involving epidermal thickening, myeloid and lymphoid infiltration, and elevated IL-23, CXCL2 and TNF[72]. The transgenic skin also shows elevated expression of endogenous IL-1F1, IL-1F5, IL-1F8 and IL-1F9, indicating an epithelial IL-1 feedback loop. IL-1F5 resembles IL-1RN, an IL-1 receptor antagonist, and the pathology in these transgenic mice is severely exacerbated when they are crossed to *Il1f5*$^{-/-}$ mice, demonstrating that IL-1F5 attenuates the proinflammatory actions of IL-1F6. Of note, human IL-1F5 and IL-1F6 are expressed at elevated levels in psoriatic skin[72].

Conversely, IL-1 cytokines also induce epithelial production of TSLP[73], which we have considered immunosuppressive or allergic inflammatory. In short, epithelial cells elaborate an intricate cocktail of IL-1 cytokines that regulates the cells' own immunoprotective competence but that also differentially activates myeloid and lymphoid responses according to the nature of the IL-1 activities present and the context. In healthy situations, the pleiotropic capacity of different IL-1 cocktails may confer balance on the resulting immune response, with some activities directly mimicking ones that operate in normal development. However, in instances of genetic or environmentally induced dysregulation, IL-1 cytokines may evoke distinct inflammatory pathologies (**Fig. 1**). For example, IL-32 (ref. 74), a recently described epithelial cytokine upregulated in chronic obstructive pulmonary disease and IBD[75,76], upregulates IL-1β secretion, TNF and IL-6. IL-32 can also synergize with NOD ligands and thus exacerbate the causative contributions of Nod2 polymorphisms to Crohn's disease[74].

In sum, the secretion by epithelial cells of TSLP, IL-25, IL-32, TGF-β and various IL-1 cytokines, including IL-1F1, IL-1F5, IL-1F6, IL-18

and IL-33, can elicit a range of functional immune outcomes that are essential to host protection, as well as to the limitation of immunopathology (**Fig. 1**). Consistent with this, defects in these epithelial pathways are increasingly associated with selective immunodeficiencies and inflammatory disease. As evidence emerges for the clinical efficacy of new anti-inflammatory agents such as those that block the IL-1 pathway, it would seem appropriate to assess such agents for their effects on epithelial-immune interactions.

Epithelial cells, B cells and IELs

The broad scope of epithelial cell influence over the immune response is further illustrated by the TLR-mediated secretion of APRIL by human colonic IECs in response to commensal bacteria. APRIL is a B cell costimulator required for immunoglobulin gene class switching to immunoglobulin (Ig) A_2, the most abundant isotype in the gut and, by mass, the most abundant immunoglobulin produced in the body[77]. In addition, TSLP induces myeloid $CD11c^+$ DCs to produce more APRIL, as well as IL-10, which also promotes class switching to IgA[78]. Conversely, several types of epithelial cells secrete SLPI (secretory leukocyte protease inhibitor), which controls the APRIL-dependent pathway, thereby limiting the levels of IgA that are induced[79]. Epithelial cells also attract B cells by their production of chemokines including CCL25 (also known as thymus-expressed chemokine, TECK), CCL28, CXCL13 (B lymphocyte chemoattractant, BLC) and CXCL12 (ref. 80). Thus, epithelial cells are key regulators of the B cell response to challenges and antigens encountered at body surfaces.

As was introduced at the start of this Review, epithelial cells also regulate the biology of IELs (see also below). All IELs require IL-15 for survival and homeostasis[5,81]. Interestingly, MyD88-deficient mice have very few IELs, and only IL-15 from the epithelial compartment can (partially) rescue this defect, strongly suggesting that IL-15 is produced by epithelial cells in response to MyD88-dependent microbe sensing[82]. As with B cells, T cells can be guided into tissues by epithelial cell chemokines: CXCL10 (also known as IP-10) and CXCL9 for type-1 T cells; CCL1, CCL22 and CCL17 for type-2 T cells[80]; and CX3CL1 for some IELs[83]. As is described in the next section, the production of such chemokines is in turn regulated by cytokines produced by immune cells, creating positive feedback loops.

Epithelial cell regulation by T cells

The capacity of DETCs and intestinal IELs to promote epithelial growth and turnover—for example, through the production of IGF1—has long been noted[84] and is not a main focus of this article. Nonetheless, we should acknowledge the existence of positively acting and negatively acting feedback loops, wherein some immunological functions of epithelial cells are themselves a response to immune cell activities. Therefore, it is appropriate that this Review briefly consider the expanding scope of epithelial cell regulation by immune cells.

The failure of $IKK\beta^{IEC\text{-}KO}$ mutant mice to mount type-2 responses to *T. muris* is associated with increased expression of IL-17A[29]. IL-6 is an obligate cofactor for IL-17A and IL-17F production by $CD4^+$ T cells[85] and can be made in large quantities by epithelial cells, which can use it as an autocrine growth factor. Thus, it is conspicuous that epithelial cells have not been strongly implicated in skewing T cells toward an IL-17-producing phenotype. Indeed, IL-17A- and IL-17F-producing cells are rare among IELs, in contrast to their high representation in subepithelial compartments of the lung and gut[86]. Conversely, IL-17A and IL-17F clearly act on epithelial cells, promoting the production of critical antimicrobial effectors in situations such as cutaneous or airway challenge by *Candida* spp.[87]. The same is true for IL-22, the receptor for which is restricted to nonhematopoietic cells such as IECs and keratinocytes. IL-22 can promote the growth of primary human keratinocytes over a 'scratch-gap' experimentally imposed on a monolayer culture. In addition, IL-22 present in supernatants of activated human T_H22 cells is necessary but not sufficient to induce keratinocytes to express increased quantities of mRNA for several immune regulators, including S100 proteins, IL-7, IL-15, IL-32, CXCL9, CXCL10, CXCL11, CCL2, CCL5, CCL20, CCL26, TLR3, TLR6 and complement components[6]. Added to this, human T_H22 cells also express several FGFs that may regulate epithelial turnover and wound healing, as has been extensively described for murine TCR-$\gamma\delta^+$ epidermal and intestinal IELs[3]. IL-22 may be produced by several tissue-associated lymphoid and myeloid cells (see above). In this regard, it was recently speculated that in the intestine, $CD11c^+$ DCs, which are abundant in the lamina propria, rapidly produce IL-22 in response to TLR activation and that IL-22's actions upon IECs upregulate CXCL10 and other chemokines that attract lymphoid IL-22-producing cells, which sustain the response[41].

Just as the effects of epithelial cytokines on immune cells are context dependent, the same is true for the effects of immune cell cytokines on epithelial cells. For example, during acute intestinal inflammation, IFN-γ and TNF synergize to promote IEC proliferation by upregulation of the epithelial β-catenin–Wnt pathway. However, as inflammation is sustained, the cytokines upregulate the Wnt-inhibitor Dkk1—inhibiting IEC proliferation, promoting apoptosis and increasing susceptibility to inflammatory pathology[88]. Again, different contexts will determine the specific cell types that produce the key cytokines. When mice are infected with the natural intestinal pathogen *Citrobacter*, lymphotoxin-β (LT-β) is essential to induce IECs to produce CXCL1 and CXCL2, which recruit neutrophils that are critical to limiting infection. Although LT-β is commonly produced by activated B cells and T cells, the critical source in this scenario is a nonadaptive, $RoR\gamma t^+$ immune cell type[89]. In sum, activated immune cell cytokines have profound effects on epithelial cells. Although this may be part of a reciprocal relationship between epithelial cells and IELs, it also reflects important bilateral cross-talk between epithelial cells and subepithelial immune cells (**Fig. 2**). Again, such localized cross-talk may prove an attractive target for clinical manipulation.

Epithelial cells costimulate

The activation of lymphoid cells usually requires at least three qualitatively distinct signals: primary activating ligands, costimulatory activating and inhibitory ligands, and cytokines and chemokines. Consistent with this, epithelial cells regulate immune cells through soluble mediators and through the interactions of epithelial cell surface moieties with receptors on immune cells (**Fig. 2**). The prototypic mediators of T lymphocyte costimulation are the B7 family members B7.1 (CD80) and B7.2 (CD86), both of which bind CD28 and CTLA-4, and which have hitherto been regarded as signatures of professional APCs, notably DCs[90]. Whereas data for epithelial cell expression of B7.1 and B7.2 are inconsistent, epithelial cells clearly can express B7-H1 (PD-L1) and B7-DC (PD-L2), which are ligands for the T cell inhibitor PD-1; B7-H2 (ICOSL), which is a ligand for the activating receptor ICOS; and B7-H3, which seems to negatively regulate target lymphocytes[91]. It will be important to link the expression of these molecules to discrete afferent signals, such as stress sensing or microbe sensing.

Meanwhile, attention has focused on more distant members of the B7 family, such as butyrophilins (Btn) and butyrophilin-like (Btnl) molecules, which are also implicated in epithelial-immune regulation. Btnl2, for example, is expressed on small intestinal epithelial cells and is upregulated in models of colitis[92]. Although target cell coreceptors

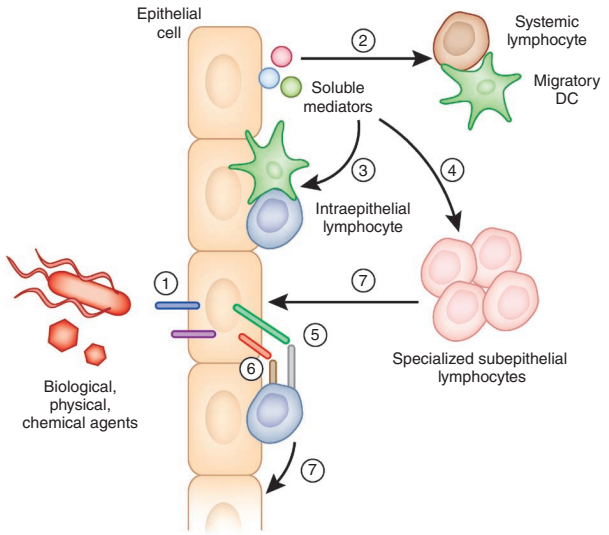

Figure 2 Molecular axes of epithelial immune cell interactions. Upon exposure to commensals, pathogens, allergens, or physico-chemical dysregulation (1), epithelial cells release antimicrobial defensins, but also send instructions by means of soluble mediators to migratory DCs and systemic lymphocytes (2), to intraepithelial lymphocytes (3) or to specialized subsets of tissue-associated subepithelial T cells (4). They also communicate with IELs by stimulatory (5) and costimulatory (6) ligand-receptor interactions. T cells reciprocate by expressing cytokines and other effectors that profoundly regulate epithelial cell biology—for example, induction of apoptosis or growth promotion (7).

for such molecules have not been identified on T cells, soluble Btnl2-Fc fusion protein can inhibit TCR-activated cell proliferation and cytokine release *in vitro*[92,93]. Thus, Btnl2 may function as a negative costimulatory molecule, limiting tissue-damaging T cell responses. Provocatively, BTN and BTNL gene polymorphisms have been associated with sarcoidosis, myositis, ulcerative colitis and tuberculosis[94–97]. Another molecule, B7S3, initially identified as a B7 family member with high homology to Btnl molecules, is also capable of inhibiting T cell activation[98]. More recently, B7S3 has been assigned to the new Btnl-like gene family *Skint*, whose members are expressed specifically in epithelia, with the potential to exert profound influence over IEL development (see below).

Epithelial–immune cell interactions are also revealing entirely new axes of T cell costimulation. For example, upregulation of the Coxsackie and adenovirus receptor (CAR) on damaged epithelial cells positively costimulates resident γδ T cells during tissue injury in the skin and intestine[99]. CAR is a member of the junctional adhesion molecule (JAM) family, which has been broadly implicated in developmental biology and inflammation. Another JAM family member, junctional adhesion molecule-like protein (JAML), is a ligand for CAR and is preferentially expressed by neutrophils, γδ T cells and some activated $CD8^+$ T cells. Epidermal and intestinal γδ T cells upregulate JAML upon coactivation with TCR-mediated signaling *in vitro* and upon tissue injury *in vivo*. Interestingly, JAML shares an intracellular phosphatidylinositol-3-OH kinase (PI(3)K) signaling motif with the classical costimulatory molecules CD28 and ICOS[100], further supporting the categorization of JAML and CAR as new, epithelium-specific costimulators.

However, perhaps the best-studied examples of costimulators displayed by epithelial cells are the MHC class I–related molecules MICA, MICB and ULBP1-5 (human) and Rae-1 isoforms α–ε, Mult-1 and H60 isoforms a–c (mouse), which are all ligands for the lymphoid activating receptor NKG2D[101]. NKG2D is expressed by many IELs, by NK cells and by some conventional $CD8^+$ T cells. Again, NKG2D engagement activates PI(3)K, in this case through an adaptor, DAP10, and very clearly can costimulate TCR-activated $CD8^+$ T cells. Of particular note, a biological context for costimulation by NKG2D ligands has been provided by our understanding of NKG2D ligand regulation. Most NKG2D ligands are inducible on epithelial cells by certain types of viral or bacterial infection and by many forms of physical chemical stress, such as osmotic shock, hyperoxidation and genotoxic events such as UV irradiation[62] (**Fig. 2**). As such, NKG2D ligands are often referred to as 'stress antigens' that contribute to rendering stressed epithelial cells targets for immune recognition. They are commonly expressed on malignant epithelial cells from many tissues, and NKG2D-deficient mice show increased susceptibility to certain types of malignancy. Likewise, DETC contribute to the resistance of mice to chemically induced squamous cell carcinoma, and when their cytolytic targeting of transformed, Rae-1^+ keratinocytes was examined, it was shown to substantively depend on NKG2D engagement[102–104]. The multiplicity of NKG2D ligands is particularly noteworthy, and not all behave in the same way. Thus, H60c is not so clearly stress responsive, being specifically and constitutively expressed by keratinocytes. In this state, it can provide positive costimulation for DETCs activated *in vitro* through the γδ TCR[105]. In sum, there is a wide variety of costimulatory molecules expressed by epithelial cells, which may reflect different means of communicating different states of epithelial cell dysregulation to target immune cells. At the same time, epithelial cells regulate their surface expression of MHC class I molecules, such as HLA-E, that suppress both NK cells and certain sets of T cells that express inhibitory counter-receptors such as NKG2A-CD94. Thus, future studies should investigate whether different combinations of positively and negatively acting costimulators evoke different responses from the lymphocytes they engage.

Epithelial cells stimulate immune cells

Epithelial cells can be induced by IFN-γ to express MHC class II, which, coupled with the capacity of these cells to digest bacteria (above), creates the potential to act as APCs. This would follow the precedent of antigen presentation to thymocytes by thymic epithelial cells. Nonetheless, experimental validation of peripheral APC activity of epithelial cells has been confounded by difficulties[106]. Conversely, epithelial cells might directly stimulate local T cells by expressing antigens for IEL TCRs. This would be consistent with the view that the intimate juxtaposition of IELs with epithelial cells and the relatively sessile nature of the IELs enforces a reliance on epithelial cells for the IEL's entire set of afferent stimuli. There is abundant experimental evidence in support of nonmicrobial stress-induced epithelial ligands for characteristically oligoclonal IEL TCRs, but as yet none has been defined. This remains an important yet hitherto frustrating area of research.

At the same time, MICA, Rae-1 and related molecules differ from other costimulators in their potential to directly activate NK cells through the NKG2D counter-receptor. Therefore, one might hypothesize that NKG2D ligand upregulation on stressed epithelial cells is sufficient to activate IELs *in vivo*. Moreover, IELs exist in an activated yet resting state[2,49], probably reflecting both their activation during development and their constitutive engagement of epithelial cells with which they are juxtaposed. This acquired state may confer a lower threshold for activation that might be satisfied by NKG2D triggering.

This hypothesis was tested by the creation of transgenic mice in which Rae-1 could be upregulated specifically in keratinocytes by a molecular switch that did not perturb the epithelial cells in any

other way[104]. The striking result was a rapid rounding of cell morphology of NKG2D$^+$ DETCs and an acquisition of activation markers. This was closely followed by similar changes in Langerhans cells, which do not express NKG2D and which were therefore responding secondarily to T cell activation. These data show that the alteration in expression of a single self-encoded stress antigen on epithelial cells can be sufficient to initiate an immune response. This process has been termed "lymphoid stress-surveillance"[107], and preliminary data suggest that it forms at least one component of the local immune response to mild epithelial trauma, such as tape stripping. It may also contribute to disease. For example, small intestinal enterocytes in celiac disease express high levels of MICA and IL-15. The IL-15 is able to upregulate NKG2D and DAP10 expression by IELs, which can then kill the MICA$^+$ enterocytes[108,109]. This direct, TCR-independent activation cannot be achieved with naive systemic T cells, again suggesting that IELs have a low threshold for at least some responses, a threshold that can be met by NKG2D engagement alone.

The expanding epimmunome

Interestingly, lymphoid stress-surveillance responses do not seem to be limited to the local environment but may promote systemic immunoglobulin responses to coadministered, adjuvant-free antigen (A.H. and J. Strid, unpublished results). The capacity of IELs activated by epithelial NKG2D ligands to promote pleiotropic functions fits with gene expression analyses of IELs that has revealed a potential for type-1, type-2, cytolytic and wound-healing responses[49,110,111]. As before, context is likely to be important. NKG2D in isolation seems insufficient to activate human NK cells *in vitro*[112] and therefore would not be expected to activate IELs. Thus, the capacity of DETCs to respond so readily to Rae-1 upregulation *in vivo* probably reflects extra, coincidental contributors to lymphocyte activation that are missing from studies *in vitro*. These may include constitutive engagement of other receptor-ligand pairs that collectively reduce the threshold for T cell activation—for example, CD103 (expressed by DETCs) engagement of E-cadherin (expressed by keratinocytes)[113]. There may be many such critical threshold-setting interactions, but we simply do not know what they are.

Threshold setting may also be a very important role of epithelial molecules in the thymus, where T cell fates are substantially determined by the magnitude of signaling through the TCR and other receptors[114]. Skint1, expressed by thymic epithelial cells, is a strong candidate for threshold setting because it is essential for the selection in the thymus of Vγ5Vδ1$^+$ thymocytes that will form the normal DETC compartment[115,116]. Without it, γδ T cells enter the epidermis but fail to express the usual TCR repertoire. In this way, Skint1 mimics the selecting effects of thymic peptide-MHC complexes on conventional T cell repertoires, although there is as yet no evidence that Skint1 engages the γδ TCR. It is noteworthy that Skint1 is the prototypic member of a large murine immunoglobulin-like superfamily, all members of which are specifically expressed by keratinocytes and by thymic epithelial cells[116]. The biological functions of the other *Skint* genes are unknown, although T cell inhibitory effects of Skint2 have been reported (above).

Likewise, there may be many other Skint-like functions exerted by as-yet unknown molecules upregulated on thymic and/or peripheral epithelial cells by the various forms of microbial and non-microbial challenge that this review has considered. Such molecules will include stress antigens but not be limited to them. Notably, they may include molecules that during immune responses reprise roles that they played in epithelial morphogenesis during development[64]. Moreover, the influence of such molecules on immune cells may be critically modified by products of the UPR and/or of other metabolic stress responses that characterize epithelial cells rapidly altering the repertoire of molecules displayed on their surface. Clearly, an ambitious but reasonable goal would be to define all the epithelial molecules that direct the actions of immune cells: we term this the 'epimmunome'. Already it includes tens of surface molecules and tens of secreted molecules, but our suspicion is that our current view of the epimmunome is akin to the mariner's view of the iceberg.

ACKNOWLEDGMENTS

We thank many colleagues for insight. Supported by the Wellcome Trust and an UK Medical Research Council program grant for psoriasis research (A.H.); the European Molecular Biology Organization and the Marie Curie Program (M.S.), and the US National Institutes of Health (C.J., W.H.).

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Published online at http://www.nature.com/natureimmunology/.
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/.

1. Anderson, G. & Jenkinson, E.J. Lymphostromal interactions in thymic development and function. *Nat. Rev. Immunol.* **1**, 31–40 (2001).
2. Hayday, A., Theodoridis, E., Ramsburg, E. & Shires, J. Intraepithelial lymphocytes: exploring the Third Way in immunology. *Nat. Immunol.* **2**, 997–1003 (2001).
3. Kronenberg, M. & Havran, W.L. Frontline T cells: γδ T cells and intraepithelial lymphocytes. *Immunol. Rev.* **215**, 5–7 (2007).
4. Laky, K. *et al.* Enterocyte expression of interleukin 7 induces development of γδ T cells and Peyer's patches. *J. Exp. Med.* **191**, 1569–1580 (2000).
5. Lai, Y.-G. IL-15 promotes survival but not effector function differentiation of CD8$^+$ TCRαβ$^+$ intestinal intraepithelial lymphocytes. *J. Immunol.* **163**, 5843–5850 (1999).
6. Eyerich, S. *et al.* Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *J. Clin. Invest.* **119**, 3573–3585 (2009).
7. Cella, M. *et al.* A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature* **457**, 722–725 (2009).
8. Buonocore, S. *et al.* Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature* **464**, 1371–1375 (2010).
9. Thorey, I.S. *et al.* The Ca2+-binding proteins S100A8 and S100A9 are encoded by novel injury-regulated genes. *J. Biol. Chem.* **276**, 35818–35825 (2001).
10. Ryckman, C., Vandal, K., Rouleau, P., Talbot, M. & Tessier, P.A. Proinflammatory activities of S100: proteins S100A8, S100A9, and S100A8/A9 induce neutrophil chemotaxis and adhesion. *J. Immunol.* **170**, 3233–3242 (2003).
11. Ghavami, S. *et al.* S100A8/A9: a Janus-faced molecule in cancer therapy and tumorgenesis. *Eur. J. Pharmacol.* **625**, 73–83 (2009).
12. Bando, M. *et al.* Interleukin-1α regulates antimicrobial peptide expression in human keratinocytes. *Immunol. Cell Biol.* **85**, 532–537 (2007).
13. Carroll, J.M., Romero, M.R. & Watt, F.M. Suprabasal integrin expression in the epidermis of transgenic mice results in developmental defects and a phenotype resembling psoriasis. *Cell* **83**, 957–968 (1995).
14. Scaffidi, P., Misteli, T. & Bianchi, M.E. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* **418**, 191–195 (2002).
15. Sims, G.P., Rowe, D.C., Rietdijk, S.T., Herbst, R. & Coyle, A.J. HMGB1 and RAGE in inflammation and cancer. *Annu. Rev. Immunol.* **28**, 367–388 (2010).
16. Kainulainen, V., Wang, H., Schick, C. & Bernfield, M. Syndecans, heparan sulfate proteoglycans, maintain the proteolytic balance of acute wound fluids. *J. Biol. Chem.* **273**, 11563–11569 (1998).
17. Bruynzeel, I., Koopman, G., van der Raaij, L.M., Pals, S.T. & Willemze, R. CD44 antibody stimulates adhesion of peripheral blood T cells to keratinocytes through the leukocyte function-associated antigen-1/intercellular adhesion molecule-1 pathway. *J. Invest. Dermatol.* **100**, 424–428 (1993).
18. Kaser, A. & Blumberg, R.S. Endoplasmic reticulum stress in the intestinal epithelium and inflammatory bowel disease. *Semin. Immunol.* **21**, 156–163 (2009).
19. Bertolotti, A. *et al.* Increased sensitivity to dextran sodium sulfate colitis in IRE1β-deficient mice. *J. Clin. Invest.* **107**, 585–593 (2001).
20. Kaser, A. *et al.* XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. *Cell* **134**, 743–756 (2008).
21. Kaser, A., Zeissig, S. & Blumberg, R.S. Inflammatory bowel disease. *Annu. Rev. Immunol.* **28**, 573–621 (2010).
22. Heazlewood, C.K. *et al.* Aberrant mucin assembly in mice causes endoplasmic reticulum stress and spontaneous inflammation resembling ulcerative colitis. *PLoS Med.* **5**, e54 (2008).
23. Kühn, R., Löhler, J., Rennick, D., Rajewsky, K. & Müller, W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* **75**, 263–274 (1993).
24. Glocker, E.O. *et al.* Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. *N. Engl. J. Med.* **361**, 2033–2045 (2009).

25. Shkoda, A. *et al.* Interleukin-10 blocked endoplasmic reticulum stress in intestinal epithelial cells: impact on chronic inflammation. *Gastroenterology* **132**, 190–207 (2007).
26. Nenci, A. *et al.* Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature* **446**, 557–561 (2007).
27. Luedde, T. *et al.* Deletion of NEMO/IKKγ in liver parenchymal cells causes steatohepatitis and hepatocellular carcinoma. *Cancer Cell* **11**, 119–132 (2007).
28. Pasparakis, M. *et al.* TNF-mediated inflammatory skin disease in mice with epidermis-specific deletion of IKK2. *Nature* **417**, 861–866 (2002).
29. Zaph, C. *et al.* Epithelial-cell-intrinsic IKK-β expression regulates intestinal immune homeostasis. *Nature* **446**, 552–556 (2007).
30. Ziegler, S.F. & Artis, D. Sensing the outside world: TSLP regulates barrier immunity. *Nat. Immunol.* **11**, 289–293 (2010).
31. Tang, H. *et al.* The T helper type 2 response to cysteine proteases requires dendritic cell-basophil cooperation via ROS-mediated signaling. *Nat. Immunol.* **11**, 608–617 (2010).
32. Kouzaki, H., O'Grady, S.M., Lawrence, C.B. & Kita, H. Proteases induce production of thymic stromal lymphopoietin by airway epithelial cells through protease-activated receptor-2. *J. Immunol.* **183**, 1427–1434 (2009).
33. Abreu, M.T. Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function. *Nat. Rev. Immunol.* **10**, 131–144 (2010).
34. Slack, E. *et al.* Innate and adaptive immunity cooperate flexibly to maintain host-microbiota mutualism. *Science* **325**, 617–620 (2009).
35. Gong, J. *et al.* Epithelial-specific blockade of MyD88-dependent pathway causes spontaneous small intestinal inflammation. *Clin. Immunol.* published online, doi:10.1016/j.clim.2010.04.001 (10 May 2010).
36. Fritz, J., Le Bourhis, L., Magalhaes, J. & Philpott, D. Innate immune recognition at the epithelial barrier drives adaptive immunity: APCs take the back seat. *Trends Immunol.* **29**, 41–49 (2008).
37. Fritz, J.H. *et al.* Nod1-mediated innate immune recognition of peptidoglycan contributes to the onset of adaptive immunity. *Immunity* **26**, 445–459 (2007).
38. Kobayashi, K.S. *et al.* Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science* **307**, 731–734 (2005).
39. Magalhaes, J.G. *et al.* Nod2-dependent Th2 polarization of antigen-specific immunity. *J. Immunol.* **181**, 7925–7935 (2008).
40. Travassos, L.H. *et al.* Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat. Immunol.* **11**, 55–62 (2010).
41. Pickert, G. *et al.* STAT3 links IL-22 signaling in intestinal epithelial cells to mucosal wound healing. *J. Exp. Med.* **206**, 1465–1472 (2009).
42. Lawrence, T., Bebien, M., Liu, G.Y., Nizet, V. & Karin, M. IKKα limits macrophage NF-κB activation and contributes to the resolution of inflammation. *Nature* **434**, 1138–1143 (2005).
43. 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).
44. Angkasekwinai, P. *et al.* Interleukin 25 promotes the initiation of proallergic type 2 responses. *J. Exp. Med.* **204**, 1509–1517 (2007).
45. Saenz, S.A., Taylor, B.C. & Artis, D. Welcome to the neighborhood: epithelial cell-derived cytokines license innate and adaptive immune responses at mucosal sites. *Immunol. Rev.* **226**, 172–190 (2008).
46. Al-Shami, A., Spolski, R., Kelly, J., Keane-Myers, A. & Leonard, W.J. A role for TSLP in the development of inflammation in an asthma model. *J. Exp. Med.* **202**, 829–839 (2005).
47. Soumelis, V. *et al.* Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat. Immunol.* **3**, 673–680 (2002).
48. Yoo, J. *et al.* Spontaneous atopic dermatitis in mice expressing an inducible thymic stromal lymphopoietin transgene specifically in the skin. *J. Exp. Med.* **202**, 541–549 (2005).
49. Shires, J., Theodoridis, E. & Hayday, A.C. Biological insights into TCRγδ$^+$ and TCRαβ$^+$ intraepithelial lymphocytes provided by serial analysis of gene expression (SAGE). *Immunity* **15**, 419–434 (2001).
50. Schmitz, J. *et al.* IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* **23**, 479–490 (2005).
51. Pastorelli, L. *et al.* Epithelial-derived IL-33 and its receptor ST2 are dysregulated in ulcerative colitis and in experimental Th1/Th2 driven enteritis. *Proc. Natl. Acad. Sci. USA* **107**, 8017–8022 (2010).
52. Perrigoue, J.G., Marshall, F.A. & Artis, D. On the hunt for helminths: innate immune cells in the recognition and response to helminth parasites. *Cell. Microbiol.* **10**, 1757–1764 (2008).
53. Zaph, C. *et al.* Commensal-dependent expression of IL-25 regulates the IL-23-IL-17 axis in the intestine. *J. Exp. Med.* **205**, 2191–2198 (2008).
54. Lajoie-Kadoch, S. *et al.* TNF-α and IFN-γ inversely modulate expression of the IL-17E receptor in airway smooth muscle cells. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **290**, L1238–L1246 (2006).
55. Hanabuchi, S. *et al.* Thymic stromal lymphopoietin-activated plasmacytoid dendritic cells induce the generation of FOXP3+ regulatory T cells in human thymus. *J. Immunol.* **184**, 2999–3007 (2010).
56. Rimoldi, M. *et al.* Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nat. Immunol.* **6**, 507–514 (2005).
57. 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).
58. Iliev, I.D. *et al.* Human intestinal epithelial cells promote the differentiation of tolerogenic dendritic cells. *Gut* **58**, 1481–1489 (2009).
59. Maynard, C.L. *et al.* Contrasting roles for all-trans retinoic acid in TGF-beta-mediated induction of Foxp3 and Il10 genes in developing regulatory T cells. *J. Exp. Med.* **206**, 343–357 (2009).
60. Kato, A. & Schleimer, R.P. Beyond inflammation: airway epithelial cells are at the interface of innate and adaptive immunity. *Curr. Opin. Immunol.* **19**, 711–720 (2007).
61. Niess, J.H. *et al.* CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science* **307**, 254–258 (2005).
62. Strid, J., Tigelaar, R.E. & Hayday, A.C. Skin immune surveillance by T cells–a new order? *Semin. Immunol.* **21**, 110–120 (2009).
63. Dinarello, C.A. The biological properties of interleukin-1. *Eur. Cytokine Netw.* **5**, 517–531 (1994).
64. Jerde, T.J. & Bushman, W. IL-1 induces IGF-dependent epithelial proliferation in prostate development and reactive hyperplasia. *Science Signal.* **2**, ra49 (2009).
65. Groves, R.W., Mizutani, H., Kieffer, J.D. & Kupper, T.S. Inflammatory skin disease in transgenic mice that express high levels of interleukin 1 alpha in basal epidermis. *Proc. Natl. Acad. Sci. USA* **92**, 11874–11878 (1995).
66. Renne, J., Schafer, V., Werfel, T. & Wittmann, M. Interleukin-1 from epithelial cells fosters T cell-dependent skin inflammation. *Br. J. Dermatol.* **162**, 1198–1205 (2010).
67. Schroder, K. & Tschopp, J. The inflammasomes. *Cell* **140**, 821–832 (2010).
68. Watanabe, H. *et al.* Activation of the IL-1β-processing inflammasome is involved in contact hypersensitivity. *J. Invest. Dermatol.* **127**, 1956–1963 (2007).
69. Dupaul-Chicoine, J. *et al.* Control of intestinal homeostasis, colitis, and colitis-associated colorectal cancer by the inflammatory caspases. *Immunity* **32**, 367–378 (2010).
70. Zaki, M. *et al.* The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. *Immunity* **32**, 379–391 (2010).
71. Takagi, H. *et al.* Contrasting action of IL-12 and Il-18 in the development of dextran sulphate colitis in mice. *Scand. J. Immunol.* **38**, 837–844 (2003).
72. Blumberg, H. *et al.* Opposing activities of two novel members of the IL-1 ligand family regulate skin inflammation. *J. Exp. Med.* **204**, 2603–2614 (2007).
73. Lee, H.C. & Ziegler, S.F. Inducible expression of the proallergic cytokine thymic stromal lymphopoietin in airway epithelial cells is controlled by NFκB. *Proc. Natl. Acad. Sci. USA* **104**, 914–919 (2007).
74. Netea, M.G. *et al.* IL-32 synergizes with nucleotide oligomerization domain (NOD) 1 and NOD2 ligands for IL-1β and IL-6 production through a caspase 1-dependent mechanism. *Proc. Natl. Acad. Sci. USA* **102**, 16309–16314 (2005).
75. Shioya, M. *et al.* Epithelial overexpression of interleukin-32α in inflammatory bowel disease. *Clin. Exp. Immunol.* **149**, 480–486 (2007).
76. Calabrese, F. *et al.* IL-32, a novel proinflammatory cytokine in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* **178**, 894–901 (2008).
77. He, B. *et al.* Intestinal bacteria trigger T cell-independent immunoglobulin A(2) class switching by inducing epithelial-cell secretion of the cytokine APRIL. *Immunity* **26**, 812–826 (2007).
78. 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).
79. Xu, W. *et al.* Epithelial cells trigger frontline immunoglobulin class switching through a pathway regulated by the inhibitor SLPI. *Nat. Immunol.* **8**, 294–303 (2007).
80. Schleimer, R.P., Kato, A., Kern, R., Kuperman, D. & Avila, P.C. Epithelium: at the interface of innate and adaptive immune responses. *J. Allergy Clin. Immunol.* **120**, 1279–1284 (2007).
81. Schluns, K.S. *et al.* Distinct cell types control lymphoid subset development by means of IL-15 and IL-15 receptor alpha expression. *Proc. Natl. Acad. Sci. USA* **101**, 5616–5621 (2004).
82. Yu, Q. *et al.* MyD88-dependent signaling for IL-15 production plays an important role in maintenance of CD8 alpha alpha TCR alpha beta and TCR gamma delta intestinal intraepithelial lymphocytes. *J. Immunol.* **176**, 6180–6185 (2006).
83. Muehlhoefer, A. *et al.* Fractalkine is an epithelial and endothelial cell-derived chemoattractant for intraepithelial lymphocytes in the small intestinal mucosa. *J. Immunol.* **164**, 3368–3376 (2000).
84. Sharp, L.L., Jameson, J.M., Cauvi, G. & Havran, W.L. Dendritic epidermal T cells regulate skin homeostasis through local production of insulin-like growth factor 1. *Nat. Immunol.* **6**, 73–79 (2005).
85. Veldhoen, M., Hocking, R.J., Atkins, C.J., Locksley, R.M. & Stockinger, B. TGFβ in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* **24**, 179–189 (2006).
86. Ivanov, I.I. *et al.* Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* **139**, 485–498 (2009).
87. Puel, A. *et al.* Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. *J. Exp. Med.* **207**, 291–297 (2010).
88. Nava, P. *et al.* Interferon-γ regulates intestinal epithelial homeostasis through converging β-catenin signaling pathways. *Immunity.* **32**, 392–402 (2010).

89. Wang, Y. *et al.* Lymphotoxin beta receptor signaling in intestinal epithelial cells orchestrates innate immune response against mucosal bacterial infection. *Immunity.* **32**, 403–413 (2010).
90. Sharpe, A.H. Mechanisms of costimulation. *Immunol. Rev.* **229**, 5–11 (2009).
91. Kim, J. *et al.* Constitutive and inducible expression of b7 family of ligands by human airway epithelial cells. *Am. J. Respir. Cell Mol. Biol.* **33**, 280–289 (2005).
92. Arnett, H.A. *et al.* BTNL2, a butyrophilin/B7-like molecule, is a negative costimulatory molecule modulated in intestinal inflammation. *J. Immunol.* **178**, 1523–1533 (2007).
93. Nguyen, T., Liu, X.K., Zhang, Y. & Dong, C. BTNL2, a butyrophilin-like molecule that functions to inhibit T cell activation. *J. Immunol.* **176**, 7354–7360 (2006).
94. Arnett, H.A., Escobar, S.S. & Viney, J.L. Regulation of costimulation in the era of butyrophilins. *Cytokine* **46**, 370–375 (2009).
95. Lian, Y., Yue, J., Han, M., Liu, J. & Liu, L. Analysis of the association between BTNL2 polymorphism and tuberculosis in Chinese Han population. *Infect. Genet. Evol.* **10**, 517–521 (2010).
96. Mochida, A. *et al.* Butyrophilin-like 2 gene is associated with ulcerative colitis in the Japanese under strong linkage disequilibrium with HLA-DRB1*1502. *Tissue Antigens* **70**, 128–135 (2007).
97. Spagnolo, P. *et al.* Analysis of BTNL2 genetic polymorphisms in British and Dutch patients with sarcoidosis. *Tissue Antigens* **70**, 219–227 (2007).
98. Yang, Y. *et al.* Characterization of B7S3 as a novel negative regulator of T cells. *J. Immunol.* **178**, 3661–3667 (2007).
99. Witherden, D.A. *et al.* Novel functional roles for JAML and CAR in epithelial γδ T cell activation. *Science* (in the press).
100. Verdino, P., Witherden, D.A., Havran, W.L. & Wilson, I.A. Molecular interaction of CAR and JAML induces recruitment of the central cell signal transducer PI3K. *Science* (in the press).
101. Eagle, R.A. & Trowsdale, J. Promiscuity and the single receptor: NKG2D. *Nat. Rev. Immunol.* **7**, 737–744 (2007).
102. Girardi, M. *et al.* Regulation of cutaneous malignancy by γδ T cells. *Science* **294**, 605–609 (2001).
103. Groh, V. *et al.* Broad tumor-associated expression and recognition by tumor-derived gamma delta T cells of MICA and MICB. *Proc. Natl. Acad. Sci. USA* **96**, 6879–6884 (1999).
104. Strid, J. *et al.* Acute upregulation of an NKG2D ligand promotes rapid reorganization of a local immune compartment with pleiotropic effects on carcinogenesis. *Nat. Immunol.* **9**, 146–154 (2008).
105. Whang, M.I., Guerra, N. & Raulet, D.H. Costimulation of dendritic epidermal γδ T cells by a new NKG2D ligand expressed specifically in the skin. *J. Immunol.* **182**, 4557–4564 (2009).
106. Hershberg, R.M. & Mayer, L.F. Antigen processing and presentation by intestinal epithelial cells—polarity and complexity. *Immunol. Today* **21**, 123–128 (2000).
107. Hayday, A.C. γδ T cells and the lymphoid stress-surveillance response. *Immunity* **31**, 184–196 (2009).
108. Meresse, B. *et al.* Coordinated induction by IL-15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. *Immunity* **21**, 357–366 (2004).
109. Hue, S. *et al.* A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease. *Immunity* **21**, 367–177 (2004).
110. Fahrer, A.M. *et al.* Attributes of gamma delta intraepithelial lymphocytes as suggested by their transcriptional profile. *Proc. Natl. Acad. Sci. USA* **98**, 10261–10266 (2001).
111. Jameson, J. *et al.* A role for skin γδ T cells in wound repair. *Science* **296**, 747–749 (2002).
112. Bryceson, Y.T., Ljunggren, H.G. & Long, E.O. Minimal requirement for induction of natural cytotoxicity and intersection of activation signals by inhibitory receptors. *Blood* **114**, 2657–2666 (2009).
113. Cepek, K.L. *et al.* Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the $\alpha^E\beta_7$ integrin. *Nature* **372**, 190–193 (1994).
114. Daniels, M.A. *et al.* Thymic selection threshold defined by compartmentalization of Ras/MAPK signalling. *Nature* **444**, 724–729 (2006).
115. Lewis, J.M. *et al.* Selection of the cutaneous intraepithelial $\gamma\delta^+$ T cell repertoire by a thymic stromal determinant. *Nat. Immunol.* **7**, 843–850 (2006).
116. Boyden, L.M. *et al.* Skint1, the prototype of a newly identified immunoglobulin superfamily gene cluster, positively selects epidermal γδ T cells. *Nat. Genet.* **40**, 656–662 (2008).