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Epithelial decision-makers : in search of the “epimmunome”

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Abstract

Frequent microbial and non-microbial 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 major decisions on innate and adaptive immune cells. Depending on context, those decisions can be as diverse as those imposed by professional antigen presenting cells, benefitting 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.

A turning tide

Epithelial cells in the thymus can initiate a spectrum of responses from progenitor thymocytes by providing tissue-restricted antigens for the T cell receptor (TCR); co-stimulatory functions, such as Notch ligands; and cytokines such as IL-7¹. In the periphery, however, the capacity of epithelial cells to initiate immune responses has been under-valued, in part because of the anatomical separation of epithelial cells from naïve T cells. Instead, the role of initiating lymphocyte responses has been passed to dendritic cells (DC) that can carry molecular information from tissues to naïve T cells in the lymph nodes. Indeed, for many years the primary contribution of body surface epithelia to host protection was viewed as physico-chemical effect or 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, based largely on two strands of evidence. First, it is increasingly clear that the direct response of epithelial cells to infection and/or stress can strongly influence DC and their subsequent regulation of adaptive responses. Second, there is emerging evidence for the direct activation by epithelial cells of lymphocyte 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. This creates the clinical potential for targeting body-surface-specific inflammatory pathways, which may prove preferable to the long-term blockade of key systemic pathways.

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Anatomical epithelial-immune integration

Epithelial cells are the major constituent of tissues lining the surface of organs or internal cavities. Consequently they are involved in a plethora of processes including the intricate regulation of secretion and adsorption in organs such as the skin, gut, and lungs, and the protection of the sub-epithelial 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 that are constructed in large part by various forms of intercellular adhesive junctions. These adhesion apparatus 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 (LC) and dendritic intra-epidermal $\gamma\delta$ T cells (DETC) 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 LC and DETC both represent tissue-restricted, self-renewing immune compartments that cannot formally be re-populated by post-natal, systemic, bone-marrow derived cells. 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 the epithelial cells may themselves function as primary sentinels.

For example, alterations in epithelial sheet structure caused by wounding, abrasion, or dysfunction of the cornification program can elicit a plethora of changes including the rapid proliferation of epithelial progenitor cells. Proteins up regulated in this hyperplastic environment include the calcium binding protein calprotectin, a nuclear heterodimer of S100A8 and S100A9 that can affect the cell's differentiation program². However, S100 proteins may also be secreted, functioning as chemo-attractants and activators of myeloid cells or lymphocytes by binding either to the immunoglobulin (Ig) super family member, RAGE, and/ or by enhancing the effects of LPS on TLR4³. Engagement of RAGE or TLR4 activates target myeloid cells and can delay their 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 anti-S100 antibodies to reduce inflammation in several models. Interestingly, S100A8 and S100A9 expression is induced by IL-1 α that is also expressed by keratinocytes⁴ (see below). Germane to this, transgenic mice overexpressing β 1 integrin in keratinocytes are characterised by high levels of IL-1 α , keratinocyte hyper-proliferation and spontaneous skin inflammation⁵ 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) that regulates chromatin, but upon release from dying epithelial cells may also engage RAGE and/or TLR4, for example on neighbouring DC⁶. RAGE activation may be strongly influenced by local oxidative, hyperglycemic and hypoxic stress, related to which, the remodelling of surface sugar moieties is another example of an epithelial cell response to barrier dysregulation⁷. For example, at the onset of epithelial trauma, heparan sulfate proteoglycans (HSPGs) are upregulated⁸. Specific keratinocyte HSPGs such as the V3 splice variant form of CD44 have been implicated in mediating close association of keratinocytes with skin T cells in the development of cutaneous inflammation⁹.

Epithelial cell dysregulation initiates inflammation

Hyper-proliferation, physico-chemical and metabolic dysregulation, and infection can each invoke a multifaceted unfolded protein response (UPR) in which the epithelial cell down-

regulates translation during attempts to resolve poor protein folding, may activate autophagy, and, failing other measures, invokes programmed cell death. Secretory epithelial cells, such as intestinal Paneth and goblet cells are particularly prone to invoking UPR, and it is now evident that beyond cell-autonomous responses, the UPR mediates interactions with immune cells¹⁰. A key UPR component is the activation from a latent state of the endoplasmic reticulum, inositol-requiring kinase/endonuclease 1 (IRE1), a highly conserved protein of which the α -subunit is ubiquitously expressed while the β -subunit is intestinal epithelial cell-specific¹¹. IRE1 cleaves 26bp from cytosolic, unspliced XBP1u mRNA to yield XBP1s, whose product transcriptionally activates major portions of the UPR.

When a mutation that blocks conversion of XBPu to XBP1s was introduced specifically into intestinal epithelial cells (IEC), the resulting mice spontaneously developed neutrophilic infiltration that variably progressed to full colitis¹². Importantly, the mice showed highly exaggerated responses, e.g over-expression of the neutrophilic chemokine, CXCL1, to two agents, TNF and bacterial flagellin, that are both commonly implicated in inflammation. These data establish the capacity of the epithelial cell 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 (UC) and Crohn's Disease (CD), the two major forms of inflammatory bowel disease (IBD)¹⁰. Likewise, the genetic association of IBD with loci encoding autophagy regulators, such as ATG16L1, probably reflects the importance of appropriate epithelial cell regulation of this related pathway.

There are many afferent activators of the UPR, and 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 imposes pressure on the UPR. Interestingly, an ethyl-nitroso-urea mutagenesis screen for colitis identified two mouse strains (*Winnie* and *Eeyore*) with mucin-2 dysregulation in goblet cells¹³. Each strain displayed high levels of ER stress, high levels of intestinal IL1 β , TNF, and IFN γ , and high propensity to inflammatory disease. Further suggesting the importance of this pathway is its regulation by IL-10, a cytokine generally regarded as immuno-suppressive. IL-10^{-/-} mice spontaneously develop colitis¹⁴, and mutations in either chain of the human IL-10R show causal association with IBD¹⁵. When added to the murine enterocyte line, MODE-K, IL-10 suppresses key components of the UPR, suggesting that its effects on epithelial cells account for some of its critical anti-inflammatory roles in the gut¹⁶.

The various cases of UPR dysregulation clearly establish the potential of the IEC to underpin inflammatory immune responses. The key questions on which this review will now focus are as follows. 1. What other pathways are implicated in the regulation of immunity by epithelial cells? 2. What are the key afferent stimuli that feed into those pathways? 3. What are the major effectors and/or cell surface-displayed molecules that communicate the state of the epithelial cell and the epithelial barrier to the immune compartment? 4. How diverse are the aggregate immune responses that result from epithelial – immune interaction? Can the consequent effects be as broad as the diversity of T cell responses induced by DC?

The central role of NF κ B

Considering Question 1, the NF κ B pathway has proved central to the integration of epithelial cells with immune effector functions. IKK γ ^{IEC-KO} mice bearing IEC-specific deletion of IKK γ /NEMO, the regulatory subunit of the IKK complex, show defects in barrier function, increased susceptibility of IEC to TNF-induced apoptosis, increased translocation of bacteria across the gut wall, and spontaneous TNF-receptor (TNFR)-dependent colitis¹⁷. The further production of TNF amplifies the pathology by causing further IEC apoptosis. This general

route to chronic inflammation is not restricted to the intestine, since hepatocyte-specific deletion of NEMO promotes spontaneous steatohepatitis, TNF-mediated liver damage, fibrosis, and the development of hepatocellular carcinoma¹⁸. Returning to the gut, IKK γ ^{IEC-KO} mice crossed to mice lacking MyD88, the principal adaptor downstream of TLRs and of the IL1-receptor (IL1R) showed no inflammation¹⁷. In relation to Question 2 (above), this result argues that TLR – mediated recognition of microbes is a common co-factor for the induction of inflammatory disease. This perspective is supported by genetic data that polymorphisms in bacterial-sensing receptors are causative in IBD (see below).

Mutations restricted to skin epithelial cells can likewise evoke inflammatory disease. When the IKK2/ β subunit, that is essential for NF κ B activation, was specifically deleted using the keratin 14 transcriptional machinery, the skin of newborn mice seemed essentially normal¹⁹. However, the mice soon developed increased epidermal thickening and focal parakeratosis, a common symptom of psoriasis, together with myeloid and lymphoid infiltration of the dermis. Cutaneous IL1 levels rose substantially, and within days, severe TNF-dependent skin inflammation developed, that was abrogated by crossing the mice to those lacking the TNF-receptor (TNFR). Thus, inflammatory responses are not invoked simply by cell-autonomous responses of keratinocytes to NF κ B dysfunction. Rather, inflammation results from a breakdown in the following pathway: environmental microbial and/or non-microbial ligands, sensed by afferent receptors on epithelial cells, trigger the NF κ B pathway, which in turn dictates the expression of gene products that maintain immune cells in an anti-inflammatory mode. Note, however, that there is no evidence to suggest that the NF κ B pathway is constitutively active in epidermal keratinocytes. Rather, it is invoked by episodic afferent stimulation.

Intestinal epithelial cells orchestrate immunity

The nature of that afferent stimulation, and of the NF κ B-dependent epithelial gene products that are induced (Questions 2 and 3, above) was clarified when the villin expression machinery was used to disrupt IKK2/ β in enterocytes²⁰. No obvious spontaneous disease developed. However, an afferent stimulus of infection by the intestinal nematode, *Trichuris muris* provoked chronic intestinal inflammation attributable to the failure of IKK2/ β ^{-/-} enterocytes to produce the IL-7-like cytokine, thymic stromal lymphopoietin (TSLP), which was recently reviewed in this journal²¹. TSLP is highly pleiotropic. It instructs neighbouring CD11c⁺ DC to upregulate OX40L and other factors that promote protective Type-2 immune responses, characterized by high levels of IL-4, IL-5, and IL-13, and the anti-parasitic effector, RELM β . Moreover it augments Type-2 cytokine production by direct effects on CD4⁺ $\alpha\beta$ T cells, and indirect effects on mast cells, NKT cells, and basophils (see also below). Thus, IKK2/ β ^{IEC-/-} mice fail to control parasite growth, and instead display highly increased levels of IL-12, IL-23, IFN γ and IL-17A/F, that promote rapid progression to severe intestinal inflammation²⁰.

This study clearly shows that the phenotypic skewing of the adaptive response, a property conventionally assigned to DC, is achieved *in vivo* by an epithelial product. However, this important study still leaves Question 2 unresolved. For example, although the IFN γ /IL-17 immune response was provoked by infection and although the IKK β -NF κ B pathway is downstream of “microbe-sensing” pattern recognition receptors (PRRs), the NF κ B pathway is likewise activated by IL-1 family cytokines (see below), 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 molecular reflections of 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 allergen²². Although the study focuses on the importance of DCs in the initiation of the immune response, it is noteworthy that subcutaneous immunization with nominal antigen and papain provoked substantial production of reactive oxygen species (ROS) by epidermal cells. This was linked to upregulation of TSLP by keratinocytes; to IL-12 downregulation by DCs; and to basophil recruitment to the draining lymph nodes, all of which promote Type-2 differentiation. Furthermore, TSLP induction depended on TLR4 (which can be engaged both by microbial moieties and by oxidized endogenous polypeptides) and its downstream signaling adaptor TRIF (“Toll-interleukin-1 receptor domain-containing adaptor inducing interferon-beta”). Collectively these data suggest that epithelial, NFκB-mediated, TLR-dependent TSLP upregulation conspires with an oxidative stress response and engagement of protease-activated receptors (e.g. PAR2) to drive adaptive Th2 responses to papain and related allergens. The consequent effects on DC and basophils may critically amplify this Type-2 skewing, rather than initiating it *de novo*.

Afferent stimuli for epithelial cells

While the respective contributions of microbial and non-microbial stress to epithelial-immune interactions have yet to be fully resolved, the “barrier-location” of epithelial cells suggests that direct microbe-sensing is a major afferent stimulus. Nonetheless, the bulk of research in this area has focused on DC, monocytes, and macrophages, and we remain uncertain of which epithelial cell subsets, in which locations, and at which point in differentiation express specific members of the seven best known classes of PRR–TLRs; NOD-like receptors (NLRs); pentraxins; mannose binding lectins (MBLs); RIG-I; and the dectin and HIN200 families. There is a developmental as well as a functional importance to understanding this, because some aspects of the microbe-sensing that is essential for the normal development of the gut associated lymphoid tissue may be attributable to epithelial cells.

TLRs

Just as is true for DC subsets, different epithelial cell subsets express different combinations of cell-surface and endosomal TLRs. Indirect evidence that IEC TLR-signalling regulates microbial penetration derives from the high levels of commensal bacteria found in the spleens of mice lacking the two adaptors, Myd88 and TRIF, that mediate all TLR signaling²³. Increased microbial penetration may chronically stimulate systemic myeloid and lymphoid cells, thereby contributing to the spontaneous inflammation that develops in aged mice with intestinal epithelial-specific suppression of the MyD88 pathway²⁴. At the same time, use of wild type bone marrow (BM) chimaerae to selectively reconstitute microbe-sensing competence in different cellular compartments of MyD88^{-/-} mice has attributed several functions to non-hematopoietic cells, e.g. epithelial cells. These range from immunoprotective production of antimicrobial effectors to immuno-suppressive effects predicted by the phenotypes epithelial cell-specific mutations in the IKK complex. For example, NFκB-mediated sensing of commensal bacteria upregulates the IL-17-related cytokine, IL-25 (IL-17E)²⁵, that selectively suppresses IL-23 production by DC (see below). Moreover, NFκB-signalling also maintains expression of IL-17Rb, the receptor for IL-25²⁶.

A major challenge now is to understand what factors determine the different outcomes of epithelial NFκB triggering. Most likely one factor will be the combinatorial context of afferent signals sensed by the epithelial cell. For example, whether or not TLR activation coincides with “stress changes” such as integrin perturbation, or with other microbial sensing pathways. Another factor most likely will be the temporal ordering of events. Thus, elegant work from Karin and colleagues has indicated that the aggregate pro-inflammatory effects of NFκB-signalling in the acute challenge phase give way to aggregate immunosuppressive, tissue-repair

responses during the subsequent resolution of inflammation. Thus, future research will need to estimate the spatial and temporal complexity of the afferent stimuli that determine epithelial cell function, that in turn instructs immune responses.

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 (eg. *H. pylori*), being generally impaired in adaptive T cell responses²⁷. Importantly, those impairments are not fully rescued by wild type reconstitution of the lymphoid and myeloid compartments. Specifically, peritoneal administration of such mice with Nod1 ligands failed to induce KC, MCP-1, and other inflammatory cytokines and chemokines, and as a result, Type-2 T cell responses were almost completely absent. Thus, pursuant to Nod1 stimulation *in vivo*, non-hematopoietic cells orchestrate a Type-2 adaptive immune response.

Strong genetic associations of Crohn's Disease (CD) have emerged for IL-23, IL-23R, HLA, and for Nod2, a bacterial-sensing PRR for muramyl dipeptide (MDP)²⁸. Besides macrophages, Nod2 shows TNF- and IFN γ -responsive expression by many human epithelial cell lines, and by primary IECs. Intestinal Paneth cells of Nod2^{-/-} mice secrete lower levels of antimicrobial defensins in response to bacterial infections, and these mice are more susceptible to oral infection by *L. monocytogenes*²⁹. Importantly, the increased susceptibility to infection is restricted to the oral route. This, together with evidence that macrophages in Nod2^{-/-} mice can still respond to and kill *L. monocytogenes*, strongly implies that the phenotype of Nod2^{-/-} mice is attributable to a defective epithelial Nod2 response. Similar to Nod1, chemokine and cytokine defects in Nod2^{-/-} mice argue that Nod2 expressed by non-hematopoietic cells promotes Type-2 T cell responses in the context of MDP and antigen³⁰.

Recently epithelial cells were also shown to form autophagosomes in response to Nod1 and Nod2 ligands³¹. In particular, MODE-K cells formed autophagosomes around intracellular *S. flexneri* bacteria, with Nod2 and ATG16L1 both recruited to the site of entry. Although fibroblasts are also capable of this, the access of IEC to luminal bacteria suggests that autophagosome formation may be a critical component of their limiting bacterial penetration. Furthermore, a capacity of epithelial cells to digest bacteria may permit them to process antigens and act *in situ* as antigen presenting cells (APC) (see below).

Diverse epithelial effectors

By channeling different afferent stimuli through key signaling pathways, epithelial cells can regulate communication with immune cells *via* the selective production of cytokines and chemokines. This goes far beyond the "default position" whereby immune cells 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: e.g. IFN γ and/or IL-17A/F- rich responses to microbial penetration; IL-4, IL-5, IL-13 and/or down- regulatory responses to abraded but not disrupted epithelia; cytolytic responses to 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 cells induce Type-2 T cell responses *via* the actions of TSLP. In addition to this, IL-25 is required for the immuno-protective Type-2 response of mice to *Trichuris muris*³². The major effects of IL-25 require an adaptive immune response, consistent with its effects on CD4⁺ T cells. However, IL-25 can also act in an autocrine fashion, promoting epithelial cell expression of TSLP. As well as contributing to host protection, TSLP and IL-25 contribute to Type-2 inflammatory diseases. Thus, mice

transgenic for TSLP in airway epithelial cells develop progressive pulmonary inflammation closely resembling human asthma³³. Atopic dermatitis (AD) patients display high cutaneous TSLP concentrations³⁴ and mice with an inducible epidermal-specific TSLP transgene develop an AD-like pathology³⁵. Moreover, we have alluded to the fact that TSLP expression is upregulated *via* protease-activated receptor 2 (PAR2), which is in turn activated by allergen-associated proteases to which Type-2 T cell responses are made³⁶.

Large amounts of IL-25 RNA have been identified in intraepithelial lymphocytes³⁷, formally qualifying conclusions about the major source of this cytokine in different scenarios. Nonetheless, the production of IL-25 by IEL 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 CD4⁺ T cells³⁸, increasing their functional differentiation, while also increasing the survival and production of Type-2 cytokines by mast cells and basophils. IL-33 is highly expressed by epithelial cells in UC, the “Type-2 form” of IBD³⁹. The formation and secretion of biologically active IL-33 secretion appears 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 a novel “alarmin”, when released in large quantities upon apoptosis or necrosis³².

Complementary to their promoting Type-2 adaptive immunity, TSLP and IL-25 are also immunosuppressive. The capacity of IL-25 to suppress IL-23 was already mentioned. TSLP inhibits the expression of IL-12 by intestinal DC, simultaneously promoting their activation of Foxp3⁺ regulatory T (T-reg) cells⁴⁰. TSLP is reportedly expressed at lower levels by colonic IEC in CD (the “Type-1 form” of IBD), whereas it is upregulated by Vitamin D3 mimetics that promote T-reg activity. Recently, Rescigno and colleagues identified a subset of human intestinal, CD103⁺ DC that seemingly adopt a tolerogenic, T-reg inducing-phenotype in response to retinoic acid (RA), TGFβ and TSLP, all produced by IEC⁴¹.

Naturally, were IEC to induce a non-responsive state in all gut DC subsets, it would be impossible to trigger adaptive immune responses to pathogens? Again, at least part of the solution lies with the epithelial cells themselves, that in response to TLR ligation secrete the chemokine CCL20 that recruits immature DC that have not been pre-conditioned⁴². Moreover, MyD88-dependent signalling in IEC induces CX3CL1 (fractalkine) that, at least in some species, promotes the DC dendrite extension into the gut lumen, where they may directly sense microbes⁴³. Nonetheless, the threshold for this shift in adaptive immunity may be high, confounding a common perception that Type-2 responses are less robust than Type-1 responses. In sum, 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 realisation evokes clinical approaches for suppressing Type-1 and/or IL-17-rich autoimmune responses, while simultaneously providing insight into the persistence of allergic responses.

IL-1 family

Conspicuous among epithelial cytokines are IL-1 family members, that bear strong evolutionary relationships to fibroblast growth factors (FGFs). IL-1α (IL1F1) can be constitutively and inducibly expressed by epithelial cells in different tissues⁴⁴. It has autocrine activity, among other things promoting production of antimicrobial peptides that limit microbial penetration and hence the level of sub-epithelial immune cell activation. At the same time, IL-1α can be pro-inflammatory, driving macrophage and T cell activation. Thus, mice transgenic for IL-1α expressed specifically by keratinocytes display dermal macrophage infiltration and focal inflammatory lesions, although without the epidermal thickening and increased proliferation associated with psoriasis⁴⁵. IL-1β (IL1F2) can be inducibly produced by epithelial cells⁴⁶. Its most intensively studied role as the primary product of the NALP-3-dependent, inflammasome-

mediated stress response, is not well-documented in epithelial cells, although emerging data identify stress-dependent NALP-3 induction in keratinocytes.

IL-1F6, F8, and F9 are largely epithelial, and keratin 14-driven IL-1F6 transgenic mice develop widespread cutaneous inflammation involving epidermal thickening, myeloid and lymphoid infiltration, and increased levels of IL-23, CXCL2, and TNF⁴⁷. The transgenic skin also showed increased expression of endogenous IL-1F1, F5, F8, and F9, indicating an epithelial IL-1 feedback loop. IL-1F5 resembles IL-1RN, an IL-1R antagonist, and the pathology in K14.IL-1F6 mice was severely exacerbated when they were crossed to IL-1F5^{-/-} mice, demonstrating that IL-1F5 attenuates the proinflammatory actions of IL-1F6. Of note, human IL-1F5 and IL-1F6 are expressed at increased levels in psoriatic skin⁴⁷.

On the other hand, IL-1 cytokines also induce epithelial production of TSLP, which we have considered as 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 activates myeloid and lymphoid responses that differ according to the nature of the IL-1 activities present and the context. In healthy scenarios, the pleiotropic capacity of different IL-1 cocktails may balance resulting immune differentiation. However, in instances of genetic or environmentally-induced dysregulation, different IL-1 cytokines evoke distinct inflammatory diseases. For example, IL-1 β secretion, together with TNF and IL-6, is induced by IL-32⁴⁸, a recently described cytokine upregulated in epithelial cells in Chronic Obstructive Pulmonary Disease and IBD^{49, 50}. IL-32 is also able to synergise with NOD ligands, and may thus exacerbate the causative contributions of Nod2 polymorphisms to CD⁴⁸. Thus, in response to Questions 3 and 4, posed earlier in this article, the secretion by epithelial cells of TSLP, IL-25, IL-32, TGF β , and various IL-1 cytokines including IL-1F1, F5, F6 and IL-33 can effect a spectrum of functional immune outcomes (Fig 1). These are essential to host protection, and to limiting immunopathology, consistent with which, defects in these epithelial pathways are associated with selective immunodeficiencies and inflammatory disease.

Epithelial cells, B cells and IEL

TLR-dependent responses of human colonic IEC to commensal bacteria induce the secretion of APRIL, a B cell co-stimulator required for Ig gene class-switching to IgA2, the most abundant isotype in the gut, and by mass the most abundant Ig produced in the body⁵¹. In addition, TSLP induces myeloid CD11c⁺ DCs to produce more APRIL, as well as IL-10 that also promotes IgA. Conversely, several types of epithelial cells secrete SLPI (secretory leukocyte protease inhibitor) which controls the APRIL-dependent pathway, thereby limiting the levels of IgA induced⁵². Epithelial cells also attract B cells by their production of chemokines including CCL25/thymus-expressed chemokine (TECK), CCL28, CXCL13/B lymphocyte chemoattractant (BLC), and CXCL12/stromal cell-derived factor (SDF)-1 α ⁵³. Thus, epithelial cells are major regulators of the B cell response to challenges and antigens encountered at body surfaces.

As was introduced at the start of this review, many epithelia feature IEL that include several types of unconventional, non-MHC-restricted T cells (see below). In the murine small intestine such cells are primarily CD8 $\alpha\alpha^+$ TCR $\alpha\beta^+$ and TCR $\gamma\delta^+$, while in the murine epidermis, >95% of DETC are TCR $\gamma\delta^+$. All require epithelial-derived IL-15 for survival and homeostasis⁵⁴. Interestingly, MyD88-deficient mice have highly reduced IEL numbers, and only IL-15 from the epithelial compartment can (partially) rescue this defect, suggesting that IL-15 is produced by epithelial cells in response to MyD88-dependent microbe-sensing⁵⁵. As for B cells, T cells can be guided into tissues by epithelial cell chemokines: CXCL10/IP-10 and CXCL9/MIG for

Type-1 T cells; CCL1, CCL22, and CCL17 for Type-2 T cells⁵³; and CX3CL1 (fractalkine) for some IEL⁵⁶.

IL-6; IL-17A/F

The failure of IKK β ^{IEC-/-} mutant mice to mount Type-2 responses to *T.muris* was associated with increased expression of IL-17A/F²⁰. IL-6 is an obligate co-factor for IL-17A/F differentiation in CD4⁺ T cells⁵⁷, and is made by epithelial cells that also use it as an autocrine growth factor. And yet, epithelial cells have conspicuously not been implicated in T cell differentiation toward IL-17, and IL-17A/F-producing cells are very rare among IEL, by contrast to their representation in sub-epithelial compartments of the lung and gut, such as the lamina propria⁵⁸. Conversely, IL-17A/F acts upon epithelial cells, promoting the production of anti-microbial effectors that seem critical in scenarios such as cutaneous or airway challenge by *Candida spp*⁵⁹. The same is also true for IL-22, which may be produced by IL-17A/F-producing T cells, and by “dedicated IL-22 T cells” found in the human dermis. Such cells also express a range of fibroblast growth factors that may regulate epithelial turnover and wound healing, analogous to the actions of DETC and intestinal IEL⁶⁰. In sum, epithelial molecules direct T cell differentiation, while activated T cell cytokines have profound effects on epithelial cells. In some cases, this reflects a reciprocal relationship between epithelial cells and IEC, but this is not always the case. That is, T cells that have major effects on epithelial cells are not necessarily those whose differentiation was determined by the epithelium.

Epithelial cells co-stimulate

The activation of lymphoid cells usually requires multiple qualitatively distinct signals. Consistent with this epithelial-immune interactions are established both by soluble mediators and by receptor-ligand interactions (Fig 2). The prototypic mediators of T lymphocyte co-stimulation are the B7-family members, B7.1 (CD80) and B7.2 (CD86) that both bind CD28 and CTLA-4, and that have hitherto been regarded as signatures of professional APC, notably DC⁶¹. Whereas data for epithelial cell expression of B7.1 and B7.2 are inconsistent, epithelial cells can express B7-H1 (PD-L1) and B7-DC (PD-L2), which are both ligands for the T cell inhibitor PD-1, B7-H2 (ICOSL), and B7-H3⁶².

More distant members of the B7 family, notably some butyrophilins (Btn) and butyrophilin-like (Btl) molecules are also implicated in epithelial-immune regulation. Btl2 is expressed on small intestinal epithelial cells and expression is upregulated in inflammatory models of colitis⁶³. Although target cell co-receptors for such molecules have not been identified on T cells, soluble Btl2-Fc fusion protein can inhibit T cell receptor (TCR)-activated cell proliferation and cytokine release *in vitro*^{63, 64}. Thus, Btl2 may function as a negative costimulatory molecule, limiting tissue-damaging T cell responses. Provocatively, BTN and BTLN gene polymorphisms have been associated with sarcoidosis, myositis, UC, and tuberculosis^{65–68}. Another molecule, B7S3, initially identified as a novel B7-family member with high homology to Btl molecules, was also capable of inhibiting T cell activation⁶⁹. More recently, B7S3 has been assigned to the newly-elucidated, Btl-like, *Skint* gene family, whose members are expressed specifically in epithelia, and can exert profound influence over IEL development (see below).

Epithelial-immune cell interactions are also revealing entirely novel axes of T cell co-stimulation. For example, recent evidence has shown that upregulation of the Coxsackie and Adenovirus receptor (CAR) on damaged epithelial cells positively co-stimulates resident $\gamma\delta$ T cells during tissue injury in the skin and intestine⁷⁰. 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, $\gamma\delta$ T cells, and

some activated CD8⁺ T cells. Epidermal and intestinal $\gamma\delta$ T cells upregulate JAML upon co-activation with TCR-mediated signaling *in vitro* and following tissue injury *in vivo*. Interestingly, JAML shares an intracellular PI3 kinase signaling motif with the classical co-stimulatory molecules CD28 and ICOS⁷¹, further supporting the categorization of JAML and CAR as novel, epithelial-specific co-stimulators.

Perhaps the best-studied examples of co-stimulators displayed by epithelial cells are the MHC I-related molecules, MICA, MICB, and ULBP1-5 (human), and Rae-1 α - ϵ , Mult-1, and H60a-c(mouse), that are all ligands for the lymphoid activating receptor, NKG2D. NKG2D is expressed by many IEL, NK cells, and some conventional CD8⁺ T cells, for which its co-stimulatory role has been established. Indeed, NKG2D blockade dramatically inhibits the diabetogenic capacity of islet-reactive CD8⁺ T cells. Interestingly, most NKG2D ligands are strikingly inducible on epithelial cells by certain types of viral or bacterial infection and by many forms of non-microbial stress, such as osmotic shock, hyper-oxidation, and genotoxic events such as u.v. irradiation⁷². As such, NKG2D ligands are often referred to as “stress-antigens”, and they are commonly expressed on malignant epithelial cells from many tissues. The DETC repertoire contributes to the resistance of mice to chemically-induced squamous cell carcinoma, and when cytolytic targeting of transformed, Rae-1⁺ keratinocytes by DETC was examined, it was shown to substantively depend upon NKG2D engagement^{73–75}. Conversely, H60c, is not so clearly stress-responsive, and is specifically and constitutively expressed by keratinocytes. In this mode, it can provide positive co-stimulation for DETC activated *in vitro* via the $\gamma\delta$ TCR⁷⁶. The multiplicity of co-stimulatory molecules expressed by epithelial cells may reflect different modes of regulation that thereby communicate different states of epithelial cell dysregulation to target immune cells. Thus, future studies should investigate whether different combinations of co-stimulators induce 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 their capacity to digest bacteria (above) creates a potential to act as APC. 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 difficulties⁷⁷. Conversely, epithelial cells might directly stimulate local T cells by express antigens for IEL TCRs. This would be consistent with the view that the intimate juxtaposition of IEL with epithelial cells and their relatively sessile nature enforces them to rely on epithelial cells for their entire set of afferent stimuli. There is abundant experimental evidence in support of non-microbial stress-induced epithelial ligands for IEL TCRs, but as yet none has been defined.

At the same time, MICA, Rae-1 and related molecules differ from other co-stimulators in their potential to directly activate NK cells *via* the NKG2D counter-receptor. Therefore, one might hypothesize that NKG2D ligand upregulation on stressed epithelial cells is sufficient to activate IEL *in vivo*. Moreover, IEL exist in an “activated-yet-resting” state³⁷, 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 way⁷⁵. The striking result was a rapid rounding-up of NKG2D⁺ DETC and an acquisition of activation markers. This was closely followed by similar changes in Langerhans cells, that do not express NKG2D, and that 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”⁷⁸, and preliminary data suggest that it forms at least one component of the local immune response to mild epithelial trauma, such as tape-stripping.

The epimmunome

Interestingly, lymphoid stress-surveillance responses do not seem to be limited to the local environment, but may promote systemic Ig responses to co-administered, adjuvant-free antigen (AH, J. Strid - unpublished). However, it is unclear whether IEL activated by epithelial NKG2D ligands can effect pleiotropic functions, or are instead limited to one type of T cell effector response. Most likely it is the former, since gene expression analysis of IEL has revealed potential for Type-1, Type-2, cytolytic, and wound-healing responses^{37, 79, 80}. As before, context will likely play a major role. A set of experiments by Ljunggren, Bryceson, Long and colleagues argue that NKG2D in isolation is insufficient to activate NK cells *in vitro*⁸¹. Therefore, the capacity of DETC to respond so readily to Rae-1 upregulation *in vivo* might reflect additional 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; e.g. CD103 (expressed by DETC) engagement of E-cadherin (expressed by keratinocytes). There may be many such “threshold-setting” interactions, but we do not know what they are.

Likewise, different types of alteration to the status of the epithelium may induce and/or suppress other ligand-receptor pairings that also influence local T cell activation. These pairings may include the *Skint* gene products, that are specifically expressed by keratinocytes and by thymic epithelial cells⁸². *Skint-1* exemplifies the profound effect that epithelial molecules can have on tissue-associated T cells, because it is essential for the selection in the thymus of V γ 5V δ 1⁺ thymocytes that will form the normal DETC compartment⁸³. Without it, $\gamma\delta$ cells enter the epidermis, but fail to display the usual TCR repertoire. In this way, *Skint-1* mimics the selecting effects of thymic peptide-MHC complexes on conventional T cell repertoires, although there is as yet no evidence that the *Skint-1* gene product engages the $\gamma\delta$ TCR. Conversely, epithelial dysregulation may induce other, as yet unidentified ligands for various IEL TCRs, as considered above. Moreover, the effects of such inducible changes may be amplified by products of the UPR, that is likely invoked in epithelial cells rapidly up-regulating stress antigens. Thus, it should be a goal to define all the epithelial molecules that direct the actions of immune cells: we term this “the epimmunome”.

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Fig 1. Epithelial cells impose diverse decisions on immune cells

Epithelial cells under various conditions express molecular mediators of immune cell instruction. Under relatively non-stressed conditions (top) the dominance of TSLP, IL-25 and other molecules shown instructs T-regulatory and Type-2 responses that, when dysregulated are associated with the diseases shown. As epithelial damage and dysregulation increases, other molecules shift the instructions to T cells toward Type-1 responses, with a different set of associated diseases. The NF κ B pathway is utilised by epithelial cells to mount both sets of responses.

Fig 2. molecular axes of epithelial immune cell interactions

Upon exposure to commensals, pathogens, allergens, or physico-chemical dysregulation (1), epithelial cells send instructions via soluble mediators to sub-epithelial DC and lymphocytes (2), to intraepithelial lymphocytes (3), or to specialised subsets of tissue-associated non-epithelial T cells such as human Th-22 cells (4). They also communicate with IEL by stimulatory (5) and co-stimulatory (6) ligand-receptor interactions. T cells reciprocate by expressing cytokines that act upon the epithelial cells (7).