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Primary immune deficiencies affecting lymphocyte differentiation: lessons from the spectrum of resulting infections

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REVIEW

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Abstract

Understanding primary immunodeficiencies has elucidated many aspects of human immunity and susceptibility to infections. Recently, defects have been identified that result in deficiencies of terminally differentiated subsets of lymphocytes including deficiencies of memory B cells, NKT cells and T_h17 T cells. Together with defects specific to T_h1 responses, these disorders revealed that dedicated pathogen-specific mechanisms exist for prevalent human pathogens, and that some host defence strategies are remarkably specific. Deficiency of T_h17 cells confirms that this subset of effector T cells is important for defence at epithelial surfaces. The clinical phenotype includes devastating complications from infection with *Staphylococcus aureus.* Since the microbial load at human epithelial surfaces is substantial and enormously diverse, this specificity could hold clues that are important for understanding first the complex symbiosis with mucosal commensals and second for understanding the consequences of manipulating these populations in inflammatory diseases.

Introduction

Infection has been the single most important determinant of the longevity of our species. Even today, infection accounts for approximately one-quarter of all human deaths, and is the most important cause of mortality in people under the age of 50 (1). Humans are unique among mammalian species in their adaptation to all terrestrial environments and therefore, the exogenous microbiota encountered is diverse. Infection clearly represents a powerful selective force shaping the evolution of immunity. However, susceptibility to infection is significant and appears to be determined to a large extent genetically. This is most obvious in the case of primary immunodeficiencies disease (PID), but applies to infection in its broader context (2).

Sterile inflammation and autoimmunity are often cited as likely countervailing selective forces. In addition, however, humans exist in symbiosis with a large number of other organisms, especially in the gut and on the skin. For example, the gut contains representatives of the three major phylogenetic kingdoms (eukaryotes, prokaryotes, archaea) and an overall load of an estimated 10^{14} organisms (3–5). This suggests a complex co-adaptation, and although this is only just beginning to be elucidated, it could represent an additional selective pressure for the specificity of host-response strategies.

Mammalian immunity is an overlay of numerous mechanisms, where phylogenetically ancient strategies of host defence act in concert with innovations; ancient biochemical (signalling) pathways are co-opted to new functions in different species and in different cell types. Many pathogens, perhaps most, are species specific, including those infecting humans (6), therefore, a complete understanding of the cellular pathways employed to adapt to these pathogens requires studies in humans. Here, we highlight how certain PIDs provide important complementary insights into some functions of human immunity and possibly clues into co-adaptation of immunity and the microbial diversity on epithelial surfaces. We start by detailing infections that predominate in patients with various PIDs that affect B cell or T cell

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differentiation and then discuss the implications for host–microbe interactions, especially host responses to commensal organisms at epithelial surfaces.

PIDs as disorders of terminal differentiation

A taxonomy of PID often begins with the important distinction between components for which antigen recognition is germ line encoded, and those for which specificity is diversified by somatic genetic recombination, and subclassified according to the component of the immune system affected. Recently, defects that compromise or completely block transcriptional programmes that specify terminal differentiation have been identified. Thus, an alternative taxonomy for PID is to divide mutations into those involving effector genes that encode functional proteins (e.g. structural proteins and enzymes) expressed by a particular differentiated cell and those that affect genes that specify terminal differentiation by virtue of binding to *cis*-regulatory elements. (See Table 1) (7). These may be influenced by signals delivered by effector molecules.

Mutations of the second type may result in less predictable phenotypes than those of the first type, not only because transcription factors may be co-opted by one or more networks that regulate developmental processes but also because components specifying terminal differentiation often vary across relatively short phylogenetic distances (e.g. between mouse and human). As a result, elucidation of these defects can reveal information about more than one cell type that cooperates to generate a specific type of immune response, or a regional compartment, such as immune responses that take place at an epithelial surface. Within the immune system, sets of functionally related effector genes have been identified in non-terminally differentiated cells, and mutations in many of these genes cause PID. For example, mutations that affect antigen receptor expression or signalling. The consequences of these defects have been reviewed recently (8, 9) and so will not be detailed here.

Terminal B cell differentiation

Terminal differentiation of B cells results in formation of memory cells and plasma cells. Human memory B cells have been further subclassified according to Ig isotype expression into unswitched (IgM^+ IgD^{lo}) and switched memory cells (IgM^- IgD^- and IgG+ or IgA+) (10). The signals that result in selective differentiation into each of these pathways remain unclear. So far, genetic defects resulting in the loss of switched memory B cells include mutations in CD40, CD40 ligand (*CD40LG*), activation-induced cytidine deaminase (*AICDA*), inducible co-stimulator (*ICOS*) and SH2 domain protein 1A (*SH2D1A*). In contrast, mutations that selectively affect the generation of unswitched memory B cells remain largely unknown (11). For instance, although there is an absence of T cell help in DiGeorge syndrome, this is not accompanied by a deficiency of unswitched memory B cells (12).

It has been proposed that the unswitched memory B-cell compartment is important for host defence against polysaccharide-encapsulated pathogens (13). This is based on a correlation between infection and deficiency of this population in common variable immunodeficiency (CVID) patients and asplenic individuals. However, this has not been a universal finding. In other cohorts of asplenic individuals, unswitched memory cells are present (14, 15), yet splenectomized individuals are highly susceptible to infection with encapsulated bacteria. Furthermore, there are experimental data which demonstrate that both unswitched and switched memory B cells are capable of eliciting protective T-cell dependent and T-cell independent (TI) immune responses following transfer into immunocompromised recipient mice

Table 1. Genetic mutations responsible for defects in lymphocyte differentiation

Terminally differentiated subset	Intrinsic effector gene defects	Phenotype	Intrinsic specification defects	Phenotype
Memory B cells	*ICOS* *TNFSF13B* *CD19*	Common variable immune deficiency	*STAT3*	Memory B cell deficiency
	SH2D1A	XLP		Memory B cell deficiency
Class-switched, high-affinity memory B cells	*CD40* *AICDA* UNG	Hyper-IgM syndrome	*NEMO/IKBKG*	Anhydrotic ectodermal dysplasia with immune deficiency
T_h1 deficiency	*IFN-γ* *IFN-γR1* *IFNγR2* *IL-12p40* *IL-12RB1* *TYK2*	MSMD	*STAT1*, *NEMO/IKBKG*	MSMD
T_h17 deficiency	*CARD9* *TYK2*		*STAT3*	AD-HIES
Treg deficiency			*FOXP3*	IPEX
NKT cells	*SH2D1A* *XIAP* *ITK*	XLP		

UNG, uracil-DNA glycosylase gene; TYK2, tyrosine kinase 2.

and immunization with either polysaccharide or protein antigens (16). Thus, additional studies are still required to definitively establish the exact and specific functions of IgM+ memory B cells in host immune responses.

Defects of terminal B cell differentiation remain among the least understood of the PIDs, although numerically they are the most significant. When associated with hypogammaglobulinaemia, the collective term CVID designates this heterogeneous group of diseases (11). There are less complete defects as well, resulting in various phenotypes that at present are classified according to analysis of circulating antibodies (17). The burden of infection in untreated CVID patients is overwhelmingly with polysaccharide-encapsulated bacteria, such as pneumococcus and Haemophilus Influenza type b. Polysaccharide-specific B cell responses in mice were defined as TI based on analysis of immune responses in CBA/N mice, a phenotype that arises from a spontaneous mutation in Bruton agammaglobulinemia tyrosine kinase (*BTK*) (18–20). In humans with *BTK* mutations, the burden of infection is also biased to polysaccharide-encapsulated pathogens, but unlike mice, most *BTK* mutations in humans result in near complete deficiency of all mature B cells (18, 21, 22).

Of effector genes, mutations affecting the BCR or its signalling apparatus usually result in defects early in B cell ontogeny, whereas those that have been identified to cause defects in terminal differentiation affect co-stimulation (8, 9). Thus, in CVID, where the naive B cell population is often close to normal in size, nevertheless, there is a similar susceptibility to polysaccharide-encapsulated organisms. The reason for the specificity of this susceptibility in antibody deficiency states remains uncertain. A small minority of CVID results from homozygous mutations that cripple ICOS (23) (Table 1). The phenotype includes disruption of germinal centre (GC) formation and loss of all switched memory B cells and the majority of unswitched memory B cells (24). More frequent defects associated with antibody deficiency are variant alleles of tumour necrosis factor receptor superfamily, member 13B [which encodes transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), a receptor for the tumour necrosis factor (TNF) ligands B cell-activating factor and a proliferation-inducing factor (APRIL); Table 1]; however, these are not consistently associated with changes in memory B cell subsets (25, 26). The association was initially attributed to a defect in signals necessary for class switch recombination, but recent mouse studies suggest TACI may act to promote plasmablast differentiation and/or survival (27). This mechanism remains to be demonstrated in humans bearing the variant TACI alleles. Furthermore, any causal association appears to be susceptible to significant modification by other genes (28).

Memory B cell formation was thought to depend on GC reactions that take place within follicles of secondary lymphoid tissues such as spleen, lymph nodes, tonsils and Peyer's patches of the gut. However, analysis of a subset of individuals with defects in GC formation (conferred by mutations in *CD40LG*, *ICOS* or *SH2D1A*) revealed that a subset of unswitched memory B cells can be generated by a mechanism that is less dependent on GCs than that underlying the generation of switched memory B cells (14, 24, 29–31). Despite these findings, there is considerable controversy whether unswitched memory B cells are derived exclusively outside of GCs (10).

The failure to generate high-affinity memory B cells and class-switched antibody also results from B cell-intrinsic defects in AICDA and uracil-DNA glycosylase, which encode the molecular machinery necessary for somatic hypermutation and class switch recombination (32, 33). Similar defects of B cell differentiation are observed in a subset of individuals with defects in inhibitor of κ, light polypeptide gene enhancer in B-cells, kinase γ (IKBKG) encoding NF-κB essential modulator (NEMO), which regulates the transcription factor NF-κB after CD40 ligation. Thus, normal activation of NF-κB is necessary for specifying terminal differentiation of B cells, although activation of NF-κB via the canonical pathway appears to be sufficient, at least under some circumstances, to induce AICDA expression (34).

Terminal memory T cell differentiation

Considerable progress has been made in identifying and understanding the transcriptional specification of terminally differentiated T cell subsets in mice and humans. In some cases, identifying monogenic mutations in cases of human PIDs that result in a complete deficiency of these subsets has elucidated their genetic specification and revealed both predicted and unforeseen phenotypes (Table 2).

T_h1 deficiency

Human deficiencies of T_h1 cells have been described in patients harbouring homozygous mutations in genes encoding IL-12p40 [interleukin 12B (*IL12B*)] or in the cytokine receptors *IL-12R*β, IFN γ receptor 1 (*IFNGR1*) or *IFNGR2*, as well as in individuals with heterozygous mutations in signal transducer and activator of transcription 1 (*STAT1*), *IFNGR1* or X-linked recessive mutations of *IKBKG/NEMO* [reviewed in (35)]. Each of these defects results in a similar infection susceptibility phenotype, dominated by low virulence and environmental Mycobacteria, *Mycobacterium tuberculosis* and typhoidal and non-typhoidal Salmonella (35). Pathological lesions in infected tissues are characterized by poorly formed granulomata (35). These defects compromise or cripple T_h1 responses, but the defects are not isolated to T_h1 cells.

Homozygous mutations in *STAT1* also results in T_h1 cells; however, the clinical presentation of these patients is more severe than those with heterozygous mutations. Thus, while partial loss of function heterozygous STAT1 mutations render affected individuals susceptible to Mycobacteria and occasionally Salmonella, complete STAT1 deficiency due to homozygous mutations is fatal due to an inability to control not only these intracellular bacterial infections but also viral infections (35, 36). This results from heterozygous mutations impairing responses to only some STAT1-activating cytokines (e.g. IFN-γ) whereas homozygous mutations abolish responses to all STAT1-activating cytokines (i.e. IFN-α/β, IFN-γ, IFN-λ1 and IL-27) (35, 36).

No humans have been identified with exon-coding defects in T-box 21 (T-bet), a master regulator of T_h1 differentiation. However, a promoter single nucleotide polymorphism

Table 2. Effects of specific gene mutations on infection susceptibility and CD4+ T cell effector function

Gene	Disease	Infection susceptibility	Affected CD4+ T cell subset
STAT1 (heterozygous) *IFN-γ/IFN-γR1/IFNγR2* *IL-12p40/IL-12Rβ* *NEMO/IKBKG*	Mendelian susceptibility to mycobacterial disease (MSMD)	Environmental Mycobacteria, Salmonella	T_h1
STAT1 (homozygous)		Mycobacterial and viral infections	T_h1
FOXP3 *CD25* *STAT5b*	IPEX IPEX like	Staphylococcus, cytomegalovirus, candida	Tregs
TYK2	AR-HIES	Virus, fungi, Mycobacteria	T_h1 T_h17 (?)
STAT3	AD-HIES	Candida, *Staphylococcus aureus*, *Haemophilus influenzae*	T_h17
CARD9	CMC	Candida	T_h17
SH2D1A *XIAP/BIRC4* *ITK*	XLP syndrome	EBV	NKT cells

TYK2, tyrosine kinase 2; *IL-12Rβ*, interleukin 12 receptor β.

appears to segregate with nasal polyposis and aspirin-induced asthma (37). Remarkably, descriptions of other infections in patients with T_h1 defects are limited to isolated case reports and appear to be rare. Thus, T_h1 cells appear to have a fundamental role in protection against Mycobacteria and Salmonella (Table 2), while a deficiency in this subset is compensated by alternative effector T cell populations that elicit efficient immune defences when challenged by the vast majority of microbial species.

T regulatory cell deficiency

Functional and numerical deficiencies of regulatory T cells (Tregs) result from mutations in the gene encoding the transcription factor forkhead box P3 (*FOXP3*), causing the *Scurfy* phenotype in mice and the immune deficiency, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome in humans [reviewed in (38); Table 2]. In both species, the phenotype is dominated by features of widespread lymphocytic infiltrates in non-lymphoid parenchyma, auto-antibody formation, colitis and atopic dermatitis (38–40). Immune dysregulation extends to extremely high levels of serum IgE that are detectable in these patients. Tregs operate in immune homeostasis and prevention of autoimmunity, and they are also selectively recruited during helminth infestations, resulting in suppression of host immunity (41).

A clinical presentation similar to IPEX has also been reported for individuals with mutations in the genes encoding CD25 (42), a component of the IL-2 receptor complex, or STAT5b (43) which is activated in response to IL-2/IL-2R signalling [reviewed in (39, 40)]. Although Tregs could develop in these individual, they exhibited functional defects due to an inability to maintain expression of FoxP3, which is known to require the IL-2R/STAT5b pathway (43) (Table 2).

While increased incidences of infections of patients with IPEX have been noted, this has been attributed to the immunosuppressive regimes employed to treat this condition (39). On the other hand, infections with opportunistic infections more associated with T-cell deficiencies (e.g. cytomegalovirus, *Candida*, *Pneumocystis*) occur in cases of CD25 (*IL2RA*) and *STAT5B* mutations, which is consistent with an important role for IL-2 and STAT5b in mediating T-cell activation in general, in addition to their specific effects in Treg biology (39, 40, 42, 43). However, some alleles of FoxP3 have been associated with abnormal hyper-responsiveness to innocuous antigens within the gastrointestinal tract (44).

T_h17 deficiency

The most recent defect identified in terminal T cell differentiation is a deficiency of T_h17 cells due to heterozygous missense mutations or microdeletions of signal transducer and activator of transcription 3 (*STAT3*), causing the clinical phenotype of autosomal dominant hyper-IgE syndrome (AD-HIES) (45–49) (Table 2). These mutations result in a dominant-negative effect including substantial reduction in expression of retinoid-related orphan receptor γt (RORγt), the transcriptional regulator of T_h17 cell generation (50).

AD-HIES is characterized by susceptibility to cutaneous and respiratory infection with *Staphylococcus aureus*; mucocutaneous candidiasis is also common. Collectively, the association between the spectrum of infection susceptibility and T_h17 deficiency in patients with *STAT3* mutations has revealed requisite functions of T_h17 cells in protection against *S. aureus* and candida infection. Despite this, the global deficiency in T_h17 cells in AD-HIES was difficult to reconcile with selective infectious susceptibility of skin and lung tissues. However, insights have recently been made to explain this. Minegishi *et al.* (51) reported that epithelial cells derived from skin (keratinocytes) or the lungs (bronchoepithelial cells) required IL-17- and IL-22-dependent signals derived from TH17 cells for their production of chemokines necessary for neutrophil recruitment and β-defensins that are important in anti-microbial immunity. In contrast, production of these effector molecules by fibroblasts, endothelial cells and macrophages could be induced by classical pro-inflammatory stimuli such as IL-1β, TNF-α and IFN-γ (51). Thus, skin and lung epithelium are strictly dependent on T_h17 products for the production of pro-inflammatory

mediators (51), which probably underlies the tissue tropism of infection that is characteristic of AD-HIES.

An important role of T_h17 cells in host defence at epithelial surfaces against superficial and dermal fungal infections is supported by the observation that partial T_h17 deficiency also occurs in chronic mucocutaneous candidiasis (CMC) (52). Strikingly, analysis of one kindred affected by CMC identified mutations in *CARD9* as the cause of this condition [(53) (Table 2)]. *CARD9* functions in myeloid cells to couple signalling pathways elicited through the C-type lectin Dectin-1 in response to fungal antigens with the production of pro-inflammatory cytokines such as IL-6 and IL-23. These cytokines subsequently guide the differentiation of naive CD4+ T cells into T_h17 effector cells (54). Thus, impaired production of IL-6 and IL-23 by Candida/Dectin-1-activated dendritic cells most likely underlies the inability to generate T_h17 cells in CMC and further highlights the critical role of T_h17 cells in mucosal immunity against commensal pathogens such as *Candida albicans*. Partial T_h17 deficiency has also been observed in patients with mutations in *IL12B* and *IL12RB1*, although these conditions have not been associated with susceptibility to infection with *S. aureus* (49).

Autosomal recessive HIES due to mutations in tyrosine kinase 2 (*TYK2*) appears to represent a more comprehensive defect in terminal differentiation, with features of both T_h17 and T_h1 deficiency (55). The clinical spectrum of infection is consistent with this conclusion, with recurrent infections with viruses, fungi and mycobacterial species. As this defect has only been identified in a single individual, detailed analysis is not available.

The mechanism underlying the extreme elevations in serum IgE levels in patients with *STAT3* mutations is unclear; however, it is unlikely to result from the T_h17 deficit since alone, since patients harbouring mutations in *IL12B* and *IL12RB1*, which precipitates a T_h17 deficiency, do not present with the elevated IgE levels that are characteristic of AD-HIES due to STAT3 deficiency (49). It also does not appear to be a consequence of B cell-intrinsic STAT3 deficiency (56). It is possible that hyper-IgE, like the distribution of infection, is the complex outcome of STAT3 deficiency in both T cells and epithelium.

NKT cell deficiency

NKT cells are a subset of lymphocytes exhibiting features of both T cells and NK cells. They are characterized by their expression of a canonical T cell antigen receptor, their recognition of glycolipid antigen presented by the non-polymorphic MHC molecule CD1d and their rapid production of effector molecules including cytokines in response to engagement of their TcR (57). NKT cells are classified as innate regulatory cells and have been implicated in protection against the development of some autoimmune conditions and malignancies (57).

An NKT cell deficiency has been described in three immunodeficient conditions that, although being caused by distinct genetic lesions, have in common impaired and often-fatal immunity against infection with EBV (Table 2). This was first described for X-linked lymphoproliferative disease (XLP) which is caused by mutations in *SH2D1A* (58) and is characterized by exquisite sensitivity to EBV infection which results in lymphohistiocytosis causing severe tissue (liver and bone marrow) damage and lymphoma (in addition to hypogammaglobulinaemia) (59). Subsequent examination of patients with XLP-like disease but normal SH2D1A sequences identified mutations in X-linked inhibitor of apoptosis (XIAP)/*BIRC4* caused a similar syndrome with respect to EBV-induced histiocytosis and an associated deficiency in NKT cells (60). More recently, homozygous mutations in *ITK* (IL-2-inducible T cell kinase) were found to be responsible for a syndrome characterized by fatal EBV-induced immune dysfunction and lymphoproliferation (61). Strikingly, the affected patients in this study also lacked NKT cells (61).

The NKT cell deficiency associated with mutations in *SH2D1A* and *ITK* was probably a primary defect because NKT cells were absent from the cord blood of one XLP patient (58) as well as gene-targetted mice lacking SH2D1A and ITK (58, 61). In contrast, it is unclear whether the NKT cell deficit in cases of XIAP/BIRC4 mutations resulted from XIAP deficiency or EBV infection (60). Irrespective of this latter uncertainty, the association between enhanced susceptibility to often-fatal EBV infection and NKT cell deficiency implies an important role for NKT cells in efficient anti-EBV immune responses in normal individuals.

Host defence at epithelial surfaces

Immune competence at epithelial surfaces is crucial, since these represent the major point of contact with microbial challenge. Barrier functions long known to be fundamental are now known to be bolstered in important ways by virtue of interactions between epithelial cells, components of the innate immune system and T cells.

On mucous membranes, the epithelial barrier is strengthened by the glycocalyx formed by the secretion and apical attachment of a heavily glycosylated mucin-rich layer by goblet cells. Together, these form a viscous and relatively impermeable sheet on the apical surface of the epithelium (62) and also provides matrix for IgA, which is abundant on epithelial surfaces. Since IgM is also abundant in epithelial secretions, this could explain the possible role for unswitched memory B cells against pathogens that enter via the respiratory mucosa. T_h17 type cytokines, particularly IL-22, appear to be crucial for the secretion of a battery of small molecules with broad-spectrum anti-microbial activity (defensins) on epithelial surfaces (63).

Spectrum of epithelial infections in PID

The infectious complications of different forms of PID provide incontrovertible evidence of the importance of specific pathways for human host defence against an organism. Overall, however, the diversity of infections observed in these defects is considerably less than those that occur sporadically or as a consequence of environmental manipulation (instrumentation, trauma, insertion of foreign body etc.). In T_h1 defects, the limited spectrum of infections (predominantly Mycobacteria and Salmonella) would not have been predicted based on findings from mice lacking T_h1 effector cells due to targeted deletion of genes including IL-12 or IL-12R, IFN-γ or IFN-γR, STAT1, STAT4 and T-bet, which

exhibit susceptibility to a very broad range of infectious pathogens from a variety of species. Consistent with evidence that T_h17 cells interact with epithelial surfaces to maintain host defences at these sites, infections in patients with absent T_h17 cells are largely confined to ectodermal locations, especially skin and lungs, and disseminated infection is relatively uncommon. The defect in epithelial defence conferred by T_h17 deficiency is also associated with a narrow spectrum of infections.

Staphylococcus aureus is a common commensal. Approximately 20% of the population is persistent carriers, and 60% are intermittent carriers. It has a predilection for the non-ciliated, non-hairy keratinized epithelium of the anterior nose (64). Streptococcal species are obligate parasites of mucosal membranes. Indeed, streptococci account for more than half of the oropharyngeal flora of humans, although most of these are from the Mitis or Salivarius groups (65). Pneumococcus is only detected in about 10–20% of adults and then comprises only a minor part of the flora. Similarly, haemophilus species are common commensals, but encapsulated type B organisms are only present in ~10% of the individuals (66).

Thus, infections encountered as a result of defects in antibody responses and T_h17 cells appear to be biased towards organisms that colonize and invade via the mouth, pharynx, respiratory tract and skin, whereas infections arising from the gut are unusual, and when they do occur, arise not from commensals but usually from parasites (*Giardia lamblia* in patients with antibody deficiency, and cryptosporidia and microsporidia species in patients with CD40 or CD154 defects) (67). This is despite the enormous diversity and abundance of enteric microbiota (10^{14} organisms with representatives from all kingdoms) and the fact that the gastrointestinal tract consists of an epithelial barrier that is only one cell thick.

Implications for immunity to commensals

In the face of this enormous microbial load, what accounts for the selectivity of the pathogen susceptibility at mucosal surfaces in patients with defects of terminally differentiated T and B cells? In particular, T_h17 cells appear to play little role in host defence in the gut despite the enormous bacterial load and their demonstrated function in regulating epithelial immunity. One possibility is that a substantial component of host defence at mucosal surfaces is independent of memory T and B cells and mediated by epithelial integrity, secretion of cytokines and microbiocides produced by epithelial cells, together with the activity of innate immune cells that provide support for these epithelial functions. Such an explanation would be consistent with the phylogenetic antiquity of epithelial–commensal relationships, which have been shown to be highly conserved among vertebrates, compared with the relatively recent development of adaptive immunity (68).

Such a scenario is also consistent with recent evidence that manipulation of epithelial function results in pathological bias of the gut microbiota. Alteration of Drosophila intestinal commensals conferred by changes in the epithelial homeobox gene, *Caudal*, which represses anti-microbial peptide production, was shown to cause lethal intestinal cell apoptosis due to the outgrowth of pathogenic bacteria (69).

Similarly, in mice, depletion of epithelial T-bet changed the balance of commensals towards those that promote colitis. This effect was independent of adaptive immunity, since it was induced in recombination activating gene-deficient mice (70). Once established in this model, colitis was transmissible by the altered commensal population to immunocompetent recipients. Thus, the epithelial response can specify the commensal population. Conversely, commensals also specify mucosal immunity. Data from experimental animals raised under gnotobiotic conditions indicate that gut commensals are necessary for the normal development of gut-associated lymphoid tissue, including well-defined T and B cell zones in Peyer's patches. Mice raised under sterile conditions also have defects in other secondary lymphoid organs (71).

Induction of gut-associated lymphoid tissue is dependent on CD4+ Lin− lineage negative cells. Development of these cells is dependent on RORγt, and it has been suggested that this population might have been selected initially to maintain mucosal integrity and later adapted and co-opted to maintain CD4+ T cell memory (72, 73). Interestingly, another innate population has been identified in the mouse gut and human mucosal-associated lymphoid tissues (74). NKp46+ $NK1.1^{dim}$ cells secrete IL-22, and their development is also dependent on commensal bacteria and RORγt. Their absence confers susceptibility to *Citrobacter rodentium* (75, 76). Taken together with human AD-HIES, these findings suggest that T_h17 cells play only a modest role in gut host defence, whereas T_h17-type cytokines appear to be crucial. By contrast, in the skin, T_h17 cells appear to have assumed a more significant role, perhaps reflecting a less abundant innate network.

These findings could have implications for understanding the relationship between commensals and induction of specific immunity to cause pathology. As noted above, commensals are necessary for normal immune development. After organogenesis is complete, exposure of epithelial cells to micro-organisms may continue to influence lymphocyte function. For example, following stimulation with bacterially derived TLR ligands, intestinal epithelial cells secrete APRIL—a ligand for TACI—which drives TI switching of human lamina propria B cells to IgA2 (77). In addition, there are intriguing data that changes in immunity, such as the induction of IgA deficiency, can influence the gut microbiota. Furthermore, changes in gut commensals have been noted in several inflammatory diseases, including colitis, arthritis and asthma, and this may represent a possible mechanism to explain the well-established but unexplained association between IgA deficiency and autoimmunity (78, 79). However, commensals are also instrumental in stimulating pathological activation of immunity.

Once the epithelium is breached, commensals are sufficient to trigger inflammatory epithelial diseases in the absence of any intrinsic defects in cells of the immune system. For example, mutations in *Filaggrin* that disrupt the keratin layer of the skin and affect hydration of the stratum corneum, resulting in clinical phenotypes of ichthyosis vulgaris and atopic dermatitis, also promote atopy, increased serum IgE, and asthma (although Filaggrin expression is

confined to the upper airway epithelium) (80–82). Similarly, a primary defect in gut mucosa is sufficient to stimulate a florid lymphocytic inflammatory response (83). In these circumstances, activation of the effector memory populations appears to play an important role in establishing and maintaining a chronic inflammatory response. PIDs provide important insights into the likely adverse consequences of antagonism of the response for therapeutic purposes

Conclusions

While PIDs that result in absolute defects in terminally differentiated CD4+ T cells or B cells have a devastating effect on longevity, this essentially results from susceptibility to a relatively narrow spectrum of infections. In particular, the human microbiota at skin and gut surfaces is substantial, and a picture is emerging of the complexity of the interactions between this micriobiota and human immunity. Recent evidence suggests that primary defects in the epithelium can alter the microbiota, to render it pathologically pro-inflammatory, and also trigger substantial bias in the nature of systemic immunity. Taken together, these findings highlight the sophistication of immune regulation, which must negotiate not only self-versus non-self but also varieties of non-self.

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Abbreviations

AD-HIES	autosomal dominant hyper-IgE syndrome
AICDA	activation-induced cytidine deaminase
APRIL	a proliferation-inducing factor
BTK	Bruton agammaglobulinemia tyrosine kinase
CMC	chronic mucocutaneous candidiasis
CVID	common variable immunodeficiency
CD40	CD40 molecule
FOXP3	forkhead box P3
GC	germinal centre
ICOS	inducible T-cell co-stimulator
IFNGR1	IFN γ receptor 1
IKBKG	inhibitor of κ, light polypeptide gene enhancer in B-cells, kinase γ
IL12B	interleukin 12B
IL12Rβ	interleukin 12 receptor β
IPEX	immune deficiency, polyendocrinopathy, enteropathy, X-linked
ITK	IL-2-inducible T cell kinase
NEMO	NF-κB essential modulator
PID	primary immunodeficiencies disease
RORγt	retinoid-related orphan receptor γt
SH2D1A	SH2 domain protein 1A
STAT1	signal transducer and activator of transcription 1
STAT3	signal transducer and activator of transcription 3
TACI	transmembrane activator and calcium modulator and cyclophilin ligand interactor
TI	T-cell independent
TNF	tumour necrosis factor
Treg	regulatory T cell
XIAP	X-linked inhibitor of apoptosis
XLP	X-linked lymphoproliferative disease

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