Organoids in immunological research

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Abstract | Much of our knowledge regarding the interactions between epithelial tissues and the immune system has been gathered from animal models and co-cultures with cell lines. However, unique features of human cells cannot be modelled in mice, and cell lines are often transformed or genetically immortalized. Organoid technology has emerged as a powerful tool to maintain epithelial cells in a near-native state. In this Review, we discuss how organoids are being used in immunological research to understand the role of epithelial cell–immune cell interactions in tissue development and homeostasis, as well as in diseases such as cancer.

Feeder cell
A cell line generated from isolated neonatal murine fibroblasts that have been selected for supporting optimal growth of epidermal keratinocytes in 2D culture.

Pluripotent stem cells
(PSCs). Cells with the potential to generate all embryonic tissues, such as embryonic stem cells.

Induced pluripotent stem cells
(iPSCs). Pluripotent cells generated in culture by the (over)expression of defined genetic factors in non-pluripotent cells such as somatic cells.

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Epithelial tissues line the boundaries of the mammalian body. For example, they are found in the skin, the gastrointestinal tract, the lungs and the thymus. Although haematopoietic cells are widely distributed throughout all tissues of the human body, the concentration of immune cells is highest in the epithelia (with the exception of the blood and lymphoid organs), where immune cells directly interact with epithelial cells[1–3]. These interactions have a role in both the maturation of immune cells (for example, in the thymus) and their activation (for example, upon wounding). Epithelial cells are the body's first point of contact with the pathogen-infested environment, and they are the first cells to respond to a pathogenic infection[4]. To safeguard homeostasis and provide a quick response to infection, epithelial cells collaborate closely with immune cells. However, epithelial cells belonging to different tissues react differently to pathogenic stimuli and, consequently, different immune cell subsets safeguard these tissues. Even within the intestine (FIG. 1a), differences in the composition of the epithelium and the microbiota along its length are mirrored by different subsets of tissue-resident immune cells[5]. The intricate interactions between the epithelium and specific components of the immune system not only are a prerequisite for preventing and containing pathogenic infections but also are important in preventing excessive immune activation, which can result in catastrophic tissue damage, as well as in the repair processes that follow tissue damage.

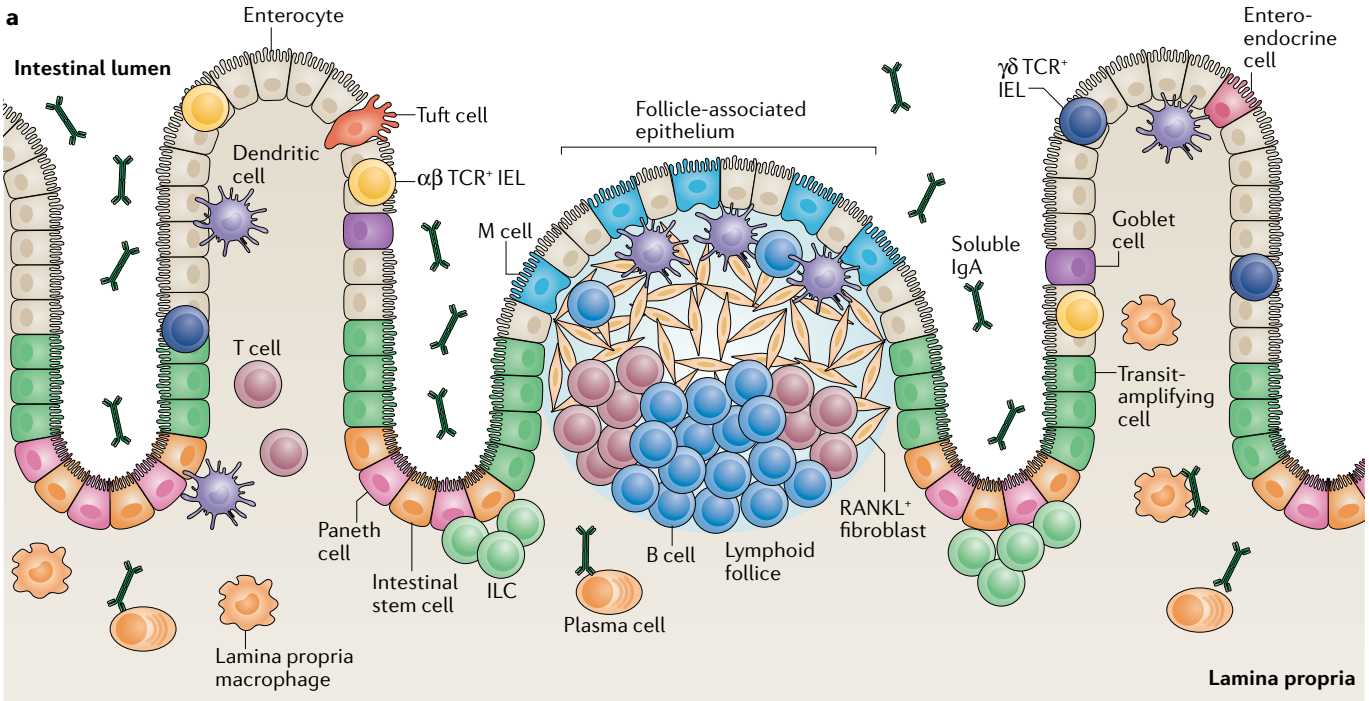
Many model systems for studying epithelial cells and their interactions with the immune system have been established over the years. Methods to culture epidermal stem cells on feeder cell layers have been used for more than 50 years[6]. Recent advances have enabled the long-term culture of adult stem cells of the intestine[7,8], liver[9–11], skin epidermis[12–14] and other epithelia[15–20] in a manner that closely mimics in vivo conditions and allows for disease modelling in vitro[21]. In the underlying approach, adult stem cells are embedded in extracellular matrix (ECM)-rich hydrogels and provided with a growth factor-defined culture medium[7,15,21]. This results in the proliferation of the epithelial stem cells into 3D cell clusters, coincident with the production of differentiated cell types. These near-native epithelial cell clusters are termed 'organoids' (BOX 1). Adult stem cell-enriched organoids can also be generated using tissue pieces composed of only epithelial cells[7] or both epithelial cells and stromal cells[8]. Organoids can also be generated from pluripotent stem cells (PSCs), either embryonic stem cells or induced pluripotent stem cells (iPSCs)[15,21]. Generally, organoids are defined as 3D cell clusters that are grown from (pluripotent or adult) stem cells and spontaneously organize into organ-like or tissue-like structures that are enriched for the cell types typically present in the tissue of origin (TABLE 1). Of relevance to this Review are organoids that represent organs in which epithelial cells have dominant functional roles, such as kidney[22], stomach[23] and intestine[24].

In contrast to feeder cell-based methods, organoid technology typically allows for long-term proliferation and differentiation of stem cells under defined (and per definition, therefore, serum-free) conditions[15,21]. These defined conditions ensure that organoid cultures are not influenced by batch-to-batch variations that can often occur in serum-based cultures. Furthermore, adult stem cell-derived organoids contain many types of (epithelial) differentiated cell that are typically present in the tissue of origin (FIG. 1b), and they are genetically stable and retain their epithelial phenotype over time[15,25]. Organoids are thus cellularly heterogeneous, in contrast to feeder cell-based cultures, which are often homogeneous. These features of organoids not only allow for a wide range of experimental applications, such as imaging, molecular analyses and gene editing[26], but also enable reductionist approaches to study the interaction of epithelial cells with immune cells. With the development of organoid models of cancer[27] and (other) immunogenic diseases such as inflammatory

bowel disease (IBD)[28], the role of immune system components in the pathogenesis of epithelial tissue diseases can be further explored.

Epithelial organoids provide a platform to study primary epithelial cells in settings that closely resemble the in vivo situation. As such, epithelial organoid cultures may also be integrated into organ-on-a-chip platforms, allowing the build-up of more complex culture systems[18,29]. Furthermore, organoids may be manipulated to invert their polarity[30], fused to form tubes that more closely resemble the architecture of the organ of origin or tissue of origin[31], or maintained as planar (2D) cultures[32–34]. Although animal models can be used to answer many questions, these models are very complex and do not permit studying direct interactions between different cell types (for example, epithelial cells and immune cells) in a reductionist manner. In addition, inherent differences between humans and mice complicate the translation of these experiments to the human situation. These considerations make epithelial organoids extremely interesting in the context of immunological research. Importantly, though, the field is still in its infancy. At least for the foreseeable future, organoid-based culture systems may need to be complemented with other approaches, such as mouse models, to capture the full extent of complex immune interactions.

In this Review, we discuss the state of the art of organoids in immunological research. We highlight the use of organoid technology to study epithelial cell–immune cell interactions during tissue development and adult homeostasis, as well as to explore the role of immune system components in tissue regeneration, inflammatory diseases and the tumour microenvironment. We also explore how organoids may be used for testing medical applications such as immunotherapy and discuss the challenges that will need to be overcome to allow these approaches to be widely used in the clinic.



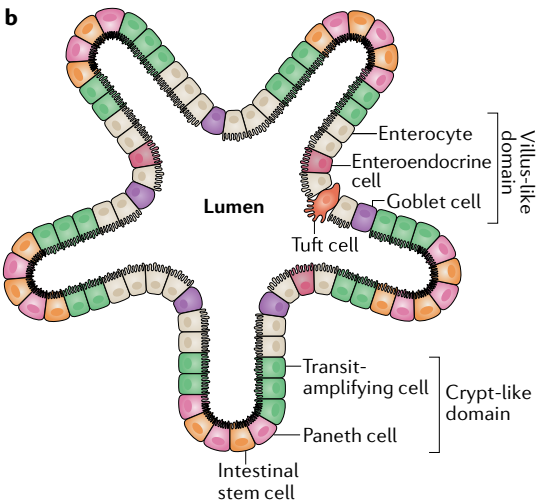


Fig. 1 | **Immune cells at the intestinal epithelial border. a** | As the epithelium and its composition differ greatly between tissues such as intestine, lung and skin, so do the tissue-resident immune cell populations. The figure provides an overview of the resident immune cells that are found at the epithelial border in the intestine at steady state. The intestinal epithelium contains αβ T cell receptor (TCR)$^+$ and γδ TCR$^+$ intraepithelial lymphocytes (IELs), whereas the intestinal lamina propria contains different subsets of dendritic cells, macrophages, T cells, IgA-producing plasma cells and innate lymphoid cells (ILCs). Along the length of the intestine, lymphoid follicles such as Peyer's patches are present, which are central locations for the differentiation of B cells to IgA-producing cells. **b** | Intestinal organoid cultures are devoid of any cellular component of the microenvironment of the intestinal epithelium. Murine small intestinal organoids spontaneously generate two specialized domains in vitro: the crypt-like domain, containing the intestinal stem cells, Paneth cells and transit-amplifying cells, and the villus-like domain, enriched for enterocytes — which make up the majority of differentiated cells in the organoids — as well as, in low numbers, enteroendocrine cells, goblet cells and tuft cells. Microfold (M) cells are not generated without treating intestinal organoid cultures with the receptor activator of nuclear factor-κB ligand (RANKL). The numbers of enteroendocrine cells, goblet cells and tuft cells detectable in the organoid culture can be further increased using a lineage-specific differentiation medium.

Box 1 | **Establishment and culture of organoids**

Adult stem cell-derived organoid cultures are often established by embedding isolated adult stem cells (when defined) or, alternatively, single-cell suspensions of the desired organ into extracellular matrix (ECM) hydrogel[14,15]. In the case of intestinal organoids, culture of crypts isolated from either the small intestine or the colon is sufficient to grow out organoids, although organoids can also be grown from isolated (flow-sorted) adult intestinal stem cells[7]. The epithelial organoid culture medium is based on a medium supplemented with growth factors relevant to the organ of interest[14,15]. For example, mouse small intestinal organoid cultures survive in medium supplemented with the growth factors noggin, R-spondin 1 or R-spondin 3, and epidermal growth factor (EGF), and the addition of exogenous WNT is often not needed, as the Paneth cells in the culture produce enough WNT for organoid propagation[7]. This is not the case for human small intestinal or colon organoids or for mouse colon organoids[126], for which exogenous supplementation of WNT is crucial for stem cell (and culture) maintenance[126]. Accordingly, organoids derived from other tissues (for example, airways, liver, pancreas, skin and urinary bladder) require that other growth factors be supplemented to the culture[14,15]. Importantly, the same holds true for tumour-derived organoids[23,126,127,151]. As tumour cells often lose dependency on some growth factors owing to genomic mutations, eliminating those growth factors from the culture medium can be used to select for a pure tumour-derived culture[22,23,127,151].

Pluripotent stem cell (PSC)-derived organoids are generated by providing culture conditions that allow for the differentiation of the PSCs into the germ layer from which the target tissue develops[14,15]. To generate intestinal organoids from PSCs, activin A is supplemented to the stem cell medium to allow for differentiation into endodermal progenitors, resulting in spheroid formation[27]. These spheroids are subsequently cultured in ECM hydrogel and medium containing the classical small intestinal organoid growth factors EGF, noggin and R-spondin 1 or R-spondin 3 (REF.[7]), triggering cell differentiation into both intestinal epithelial cells and mesenchymal cells[14,15,27].

Studying fetal development

As mouse peripheral tissues are not colonized by adaptive lymphocytes until after birth[35–37], other systems are needed to model the interaction of human fetal intestines with cells of the immune system. Therefore, organoid cultures have been generated from mouse and human fetal intestinal epithelium to study epithelial cell–immune cell interactions in fetal development[38]. For example, a recent study identified a population of $CD4^+CD69^+$ effector memory T (T_{EM}) cells that produce tumour necrosis factor (TNF) and express genes promoting epithelial cell growth and proliferation[39]. This study used human fetal intestinal organoids to show that low doses of TNF support the proliferation of fetal intestinal stem cells (ISCs), whereas high levels of TNF induce ISC apoptosis and thereby prevent the expansion of fetal ISC populations. In line with this observation, organoid co-cultures with low numbers of autologous fetal T_{EM} cells enhanced organoid growth, an effect that was blocked by simultaneous TNF inhibition. Conversely, organoid growth was reduced when co-cultured with fetal T_{EM} cells at high concentrations, which could also be reversed by blocking TNF[39]. This organoid system was used to model the development of necrotizing enterocolitis in babies born preterm, who have greater numbers of intestinal TNF-producing T_{EM} cells than do babies born at term[39]. This study shows that disease-relevant interactions can be readily studied using organoids from fetal tissues.

Studying adult homeostasis

As mentioned above, epithelial cell–immune cell interactions have an important role in mammalian homeostasis, including immune cell development, as well as maintaining homeostasis at border tissues such as the skin and the intestine. Here we discuss the role of organoid culture systems in studying these homeostatic interactions.

Thymic organoids to study T cell development. The thymus is the central location of T cell maturation and final differentiation from progenitor cells to mature naive lymphocytes[40]. T cell progenitors originating from the bone marrow undergo positive selection in the thymic cortex and subsequent negative selection in the thymic medulla. These regions of the thymus consist of two separate populations of epithelial cells: cortical thymic epithelial cells (cTECs) and medullary thymic epithelial cells (mTECs)[41].

Re-creating the 3D environment of the thymus has been shown to be crucial for modelling its function[35,36]. To study T cell development in a near-native environment, several methods for the generation of thymic organoids have been described[42–45]. These organoid cultures have generally been established from human or mouse fetal or neonatal thymic tissue, but there are also reports of the in vitro differentiation of TEC-like cells from human embryonic stem cells[46,47]. These cultures all resulted in thymus-like structures that generated viable T cells in vitro and were functional upon transplantation to nude mice. Interestingly, although prolonged thymus-like cultures were possible (up to 56 days in vitro[44]), the cells lost colony-forming capacity upon serial passaging[48]. Therefore, whereas short-term assays can be carried out in currently available systems, further optimization of the cultures will be required for longer-term studies. Importantly, although a bi-potent TEC precursor has been described in adult mice (albeit there is some controversy about that matter in the field[49,50]), thymic organoids containing both cTECs and mTECs have not yet been generated from a single stem cell. From a developmental biology perspective, it would be interesting to see whether a human TEC precursor can be identified. Also, given that some of the growth factor requirements of the bi-potent TEC precursors in mice have been described (for example, BMP4 and IL-22)[51,52], it would be interesting to see whether it is feasible to maintain TEC progenitor-derived organoids. Finally, because loss of thymic function occurs in various pathological conditions[53,54], dissecting the interactions between the thymic epithelium and immune cells will be of interest.

Epithelial cells as immune sentinels for bacteria. Epithelial cells are essential components of the body's defence against invading pathogens and therefore express various pattern recognition receptors that recognize pathogens[55]. As the microbiota differs in different epithelial tissues, so does the pathogen recognition machinery that is expressed by epithelial cells, even within the same organ. For example, it has been shown that epithelial cells from different segments of the gut (small intestine versus colon) have different patterns of Toll-like receptor (TLR) expression[56]. Organoids that were produced from these different anatomical locations retained this expression pattern in culture, as well as the associated changes in transcriptional signature that occur downstream of

Intestinal stem cells
(ISCs). Rapidly dividing, $LGR5^+$ columnar crypt bottom cells from which all cells in the intestinal epithelium arise.

Necrotizing enterocolitis
A condition that typically occurs in newborns, in which a part of the intestine dies.

Positive selection
The process leading to the proliferation and survival of thymocytes that have successfully recombined the T cell receptor locus to express a functional T cell receptor on their cell surface.

Negative selection
The process leading to clonal deletion of thymocytes that express a T cell receptor that binds to self-peptide–MHC complexes presented in the thymic medulla.

Nude mice
Mice that are athymic (lacking a thymus) and therefore lack mature, functional T cells and are severely immune deficient.

Table 1 | **Characteristics of different organoid systems**

Model	Features	Advantages	Disadvantages	Refs
Organoid models of development, homeostasis and non-cancer diseases				
Adult stem cell-derived organoids	Contain epithelial cells (including stem cells, progenitor cells and differentiated cells)	Capture interpatient genetic heterogeneity; cultures can be expanded and passaged for extended periods; culture medium can be altered to allow for lineage selection towards particular subtypes of differentiated cells; amenable to genetic manipulation; pure epithelial cell culture	Do not contain all cellular components of the tissue microenvironment, including fibroblasts, endothelial cells and immune cells; access to tissue may be difficult or limited; apical side is not exposed	7,9–12,14, 16–20,29
Organoids derived from tissue pieces	Contain epithelial cells and mesenchymal cells	Capture interpatient genetic heterogeneity; contain all cellular components of the tissue microenvironment	Access to tissue may be difficult or limited; limited culture (at least 30 days, in some cases up to 1 year)	8
Pluripotent stem cell-derived organoids	Contain epithelial cells and mesenchymal cells	Well-established culture protocols for many tissues; long-term culture with progressive differentiation	Impure cell culture; do not capture intrapatient genetic heterogeneity; ethical issues, in particular when using embryonic stem cells	22,24
Organoids used in planar cultures	Contain epithelial cells (including stem cells, progenitor cells and differentiated cells)	Both apical and basal sides are exposed	Cellular composition or cell behaviour may change owing to different culture conditions (switch from 3D to 2D)	18,32,33
Inverted organoids	Contain epithelial cells (including stem cells, progenitor cells and differentiated cells)	Apical side is exposed; extended culture period	Do not contain all cellular components of the tissue microenvironment, including fibroblasts, endothelial cells and immune cells; access to tissue may be difficult or limited	30
Organoids used in organ-on-a-chip models	Contain epithelial cells (including stem cells, progenitor cells and differentiated cells)	Capture interpatient genetic heterogeneity; culture medium can be altered to allow for lineage selection towards particular subtypes of differentiated cells; pure epithelial cell cultures in tissue-like topography; apical side is exposed	Access to tissue may be difficult or limited; limited culture period	18,29
Organoid models of cancer				
Organoids derived from epithelial tumour cells	Contain epithelial tumour cells	Resemble histology of the tumour epithelium; may preserve cellular and genetic heterogeneity of the tumour epithelium in situ; expansion and long-term culture also on a clonal level are possible	Include only tumour epithelium; do not model tumour immune editing (the clonal selection of tumour cells by immune escape)	135–137
Tumour cells cultured in air–liquid interface (holistic approach)	Contain (epithelial) tumour cells and cells of the tumour microenvironment	May contain various cellular components of the tumour microenvironment	Limited culture period	143,146
Organotypic tumour spheroids	Contain (epithelial) tumour cells and cells of the tumour microenvironment	Include entire tumour microenvironment	Limited culture period	144,145

the different TLRs following challenge with microbial products[56,57]. These data imply that basal TLR expression patterns in the intestine are independent of both the microbiota and immune cells. However, although stimulation of organoids with TLR ligands induced a specific antimicrobial gene-expression signature, antimicrobial peptides were not induced by TLR stimulation but rather by immune cell-secreted cytokines[56]. Indeed, although TLR5 is expressed by Paneth cells[56], interferon-γ (IFNγ), but not the TLR5 ligand flagellin, induced Paneth cell degranulation and extrusion[57]. These observations highlight the complexity of the tripartite interaction between the epithelium, commensal microbiome and immune system. To complicate matters further, additional cell types, such as stromal cells underlying the epithelium[58] and enteric neurons, influence both epithelial function and the immune system[59].

Immune compounds that influence epithelial cell differentiation. Co-culture systems of epithelial organoids with different cell types surrounding the epithelium allow these complex interactions to be studied in vitro. Several such studies have recently shown that immune cell-derived cytokines and the microbiome markedly influence the differentiation of intestinal epithelial cells[60–62] (FIG. 1). The clearest example in this respect is that of microfold (M) cells, which are highly transcytotic cells present in the follicle-associated epithelium

(FAE) that overlays mucosa-associated lymphoid tissues (MALT), such as intestinal Peyer's patches and similar tissues in the airways[63]. M cells have an important role in inducing immune responses, by trafficking luminal content to haematopoietic cells residing underneath the FAE. Mice lacking M cells have decreased production of secretory IgA and dysbiosis[58,64,65]. In the intestine, M cells are derived from LGR5$^+$ ISCs. M cell differentiation requires stimulation with the receptor activator of nuclear factor-κB (NF-κB) ligand (RANKL; also known as TNFSF11), which is expressed on stromal cells adjacent to the FAE[58], resulting in expression of the early M cell marker SPIB[61,66,67]. No M cells are present in intestinal organoids in the absence of RANKL, which shows that this external stimulus is required to push ISCs and transit-amplifying cells into an M cell fate (FIG. 1b).

M cells are extremely rare, comprising only ~10% of the FAE, which in turn comprises a very small part of the intestinal epithelium. Similarly, tuft cells and enteroendocrine cells comprise only a minor fraction of the cells in the small intestine (FIG. 1). In general, culture of organoids with defined media has enabled the enrichment of these rare cell types in vitro[62,68–71], such that they could be thoroughly characterized. In the specific case of M cells, RANKL-treated organoids were used to establish a transcriptional signature of these cells[72]. This signature was subsequently used to identify M cells within cell populations sorted directly from the intestinal epithelium. Furthermore, it was confirmed that M cell differentiation depends on TNF receptor-associated factor 6 (TRAF6)-mediated RANKL signalling, which leads to downstream NF-κB signalling, with the subsequent transcription of *SPIB*[73]. Also, it was shown that expression of the mature M cell marker GP2 is enhanced in differentiating M cells by RANKL-dependent expression of the transcription factor SOX8 (REF.[74]). Organoid studies have sometimes produced results that were not expected from in vivo experiments. For example, it was assumed on the basis of in vivo experiments that IL-22-induced signalling in the FAE leads to a decrease in the number of M cells. Indeed, an absence of IL-22-binding protein (IL-22BP) expressed by tissue-resident dendritic cells (DCs) (and, therefore, increased IL-22-induced signalling) leads to decreased uptake of luminal antigens in Peyer's patches. However, the addition of IL-22 to RANKL-stimulated organoids did not alter the expression levels of M cell-associated genes[75]. This showed that IL-22-induced signalling does not influence M cell differentiation directly, and that the reduced antigen uptake in IL-22BP-deficient mice was a secondary effect of IL-22-induced signalling in Peyer's patches that was independent of M cell differentiation. As is shown by this last example, the use of organoids instead of classical in vivo experiments has helped dissect the direct response of the epithelium to stimuli provided by other cell types residing in the same niche.

Immune cell-derived cytokines have also been shown to influence epithelial cell differentiation in other organoid settings. For example, IL-13 has been shown to induce the differentiation of goblet cells in mouse intestinal organoids, and epithelium-secreted IL-33 was shown to enhance IL-13 production by group 2 innate lymphoid cells (ILC2s)[76]. Furthermore, IL-13 and IL-4 have been shown to induce tuft cell differentiation[62,70,71]. In the case of tuft cells, the interplay between the epithelium and the immune system is particularly interesting. In vivo, tuft cells have been described as cells expressing chemosensory receptors in mammalian intestine and airways[77]. In the intestine, tuft cells also function as immune sentinels that react to the presence of parasites. For example, helminth-derived succinate activates tuft cells to secrete the alarmin IL-25 (REFS[62,70,71,78,79]). Similarly to IL-33, IL-25 recruits ILC2s and stimulates these cells to produce IL-4 and IL-13, leading to goblet cell hyperplasia, enhanced mucus production and subsequent expulsion of parasitic worms. In intestinal organoids, co-culture with activated ILC2s, as well as stimulation with either IL-4 or IL-13, leads to increased numbers of tuft cells[62,70,71]. Organoid treatment with IL-4 and IL-13 or co-culture with pre-activated T helper 2 (T_H2) cells (which produce these cytokines) caused a concomitant decrease in the number of ISCs, as would be expected in organoids that are pushed towards differentiation[60]. Likewise, the addition of other pro-inflammatory T_H cell-derived cytokines, such as IFNγ and IL-17, or co-culture with pre-activated T_H1 cells and T_H17 cells, gave rise to the same relative decrease in the number of ISCs, as well as to increased numbers of transit-amplifying cells. Although T_H2 cell-derived cytokines induced tuft cell differentiation, there was no apparent increase in the frequencies of other fully differentiated cells (such as enterocytes) in IFNγ-treated or IL-17-treated organoids. Conversely, treatment with the anti-inflammatory cytokine IL-10 or co-culture with peripherally induced regulatory T cells increased the number of ISCs[60]. Interestingly, IL-22, a member of the IL-10 family of cytokines, which is mainly secreted by T_H cells and group 3 innate lymphoid cells (ILC3s), induced stem cell proliferation and subsequent growth of the organoid through signal transducer and activator of transcription 3 (STAT3) signalling[80]. Similarly, IL-2 derived from activated T cells enhanced the growth of human iPSC-derived intestinal organoids, as well as the expression of markers of mature intestinal epithelial cells, reportedly through STAT3 signalling[81]. Importantly, the latter observation could also depend on STAT5 signalling, which can be triggered by the IL-2 receptor[82] and has been shown to be important in inducing Paneth cell differentiation and tissue regeneration upon intestinal damage[83].

Interactions with the epithelium that influence immune cell phenotype. The studies described in the previous section show that relatively simple assays can be extremely informative as to the direct effects of immune cell-derived factors on the epithelium (FIG. 2). However, the reciprocal interplay between the immune system and the epithelium that drives mucosal immunity is not only mediated by soluble factors. A subset of intraepithelial lymphocytes (IELs) resides between epithelial cells in various tissues, such as tissue-resident T cells in the intestine and other tissues[84–92]. These CD4$^+$ and CD8$^+$ T cells either develop in the thymus ('natural' IELs) or are tissue-resident memory cells that remain

Tuft cells
A subset of chemosensory cells in the intestinal and airway epithelium.

Enteroendocrine cells
A group of specialized hormone-producing intestinal epithelial cells.

Goblet cells
A subset of mucus-producing cells found in the intestinal and airway epithelium.

Group 2 innate lymphoid cells
(ILC2s). A subset of innate lymphocytes (that is, lymphocytes that do not express an antigen receptor) that produce type 2 cytokines (such as IL-5 and IL-13) upon stimulation.

Peripherally induced regulatory T cells
Regulatory T cells that differentiate from naive T cells in the periphery, as opposed to naturally occurring regulatory T cells that develop in the thymus.

Group 3 innate lymphoid cells
(ILC3s). A subset of innate lymphocytes (that is, lymphocytes that do not express an antigen receptor) that produce T_H17 cell-associated cytokines (such as IL-17A and IL-22) upon stimulation.

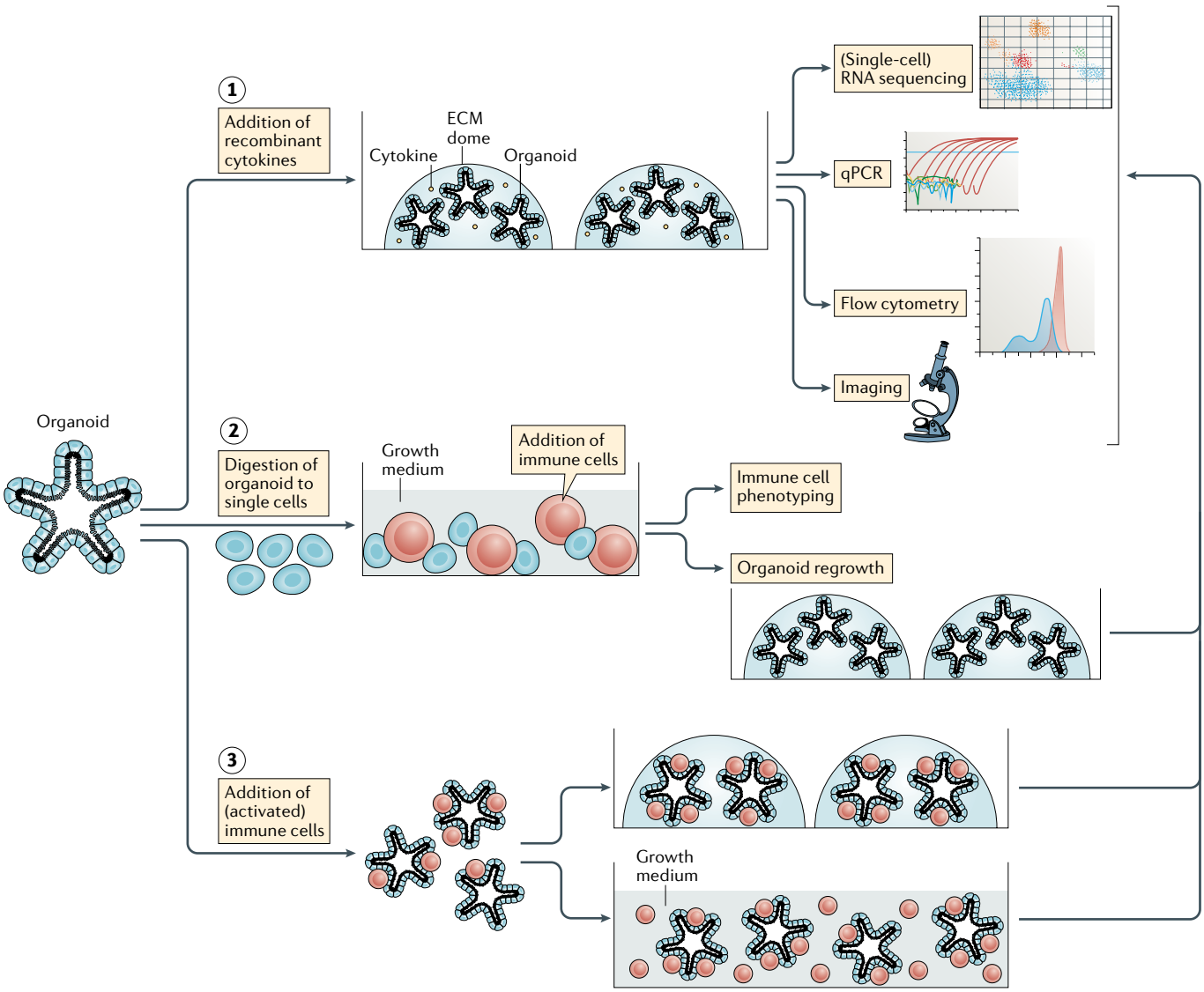


Fig. 2 | **Organoid–immune cell co-culture systems in basic research for the study of interactions between immune cells and the epithelium.** Several co-culture systems of organoids with immune cells and/or their products have been developed. (**1**) Treatment of organoids with recombinant cytokines in extracellular matrix (ECM) domes is used to assess the influence of immune cell-derived cytokines on epithelial cells. For example, IL-4 and IL-13 enhance tuft cell differentiation, whereas IL-22 supports stem cell proliferation and survival. (**2**) Digestion of organoids to single cells and their subsequent regrowth in the presence of immune cells is similarly used to assess the influence of immune cells and immune cell-derived cytokines (either soluble or membrane bound) on organoid growth and differentiation, as well as the effects of epithelial cells on immune cell phenotype. (**3**) The addition of (activated) immune cells, such as T cells or innate lymphoid cells (ILCs), to intact organoids in ECM domes or in growth medium (suspension culture) is used to assess the reciprocal interactions between immune cells and epithelial cells. The readout of these assays typically involves digestion of the formed organoids and subsequent transcriptomics (either (single-cell) RNA sequencing or quantitative PCR (qPCR)), imaging and/or flow cytometry-based assessment of epithelial cell and/or immune cell phenotypes. The immune cells used in these co-cultures either are sorted directly from mouse tissues (for example, splenocytes, intestinal intraepithelial lymphocytes, lamina propria T cells, lamina propria ILCs or pulmonary ILC2s) or are derived from human peripheral blood, either directly or following in vitro differentiation.

in the tissue after infection and subsequent resolution (known as 'induced' or 'peripheral' IELs). In mice, proximity to the epithelium seems to be sufficient by itself to change the phenotype of T cells[84]. Indeed, co-cultures of mouse small intestinal organoids supplemented with cytokines necessary for T cell survival supported the proliferation and maintenance of both αβ IELs and γδ IELs[92]. In addition, in vitro-activated and naive splenic $CD8^+$ T cells specific for ovalbumin (OVA) adopted an IEL-like phenotype upon co-culture with intestinal organoids expressing OVA-derived peptides[93], which shows that antigen-specific interactions are involved in this differentiation process.

Recently, it was shown that an ISC subset in mice can function as non-classical antigen-presenting cells that present antigens to $CD4^+$ T cells in the context of

MHC class II molecules. Through antigen presentation, these ISCs activate lamina propria-resident CD4$^+$ T cells to produce various T_H cell-derived cytokines, which then influence epithelial cell differentiation[60]. Interestingly, whereas co-culture of intestinal organoids with antigen-specific naive CD4$^+$ T cells did not seem to elicit activation of the T cells[93], T cells co-cultured with sorted ISCs were activated[60]. This discrepancy could be explained by the relatively low number of ISCs in organoid cultures, which thereby reduces the sensitivity of this particular assay.

Many questions regarding the effects of epithelial cells on specific immune cell subsets remain unanswered. For example, at least in the skin, there are clear differences in the migration and homing patterns of tissue-resident T cells after infection with herpes simplex virus[94], and differences in behaviour were also shown between intestinal αβ IELs and γδ IELs[85]. In the intestine, the migratory behaviour of IELs into the epithelium and along the villi depends on the microbiota[95,96]. At least for γδ IELs, the microbiota-induced cues are provided via the epithelium, as this migratory behaviour of γδ IELs was not seen in *Myd88*$^{-/-}$ mice, which lack microorganism sensing in the intestinal epithelium[96]. To shed light on this tripartite interaction between epithelial cells, immune cells and the microbiota, and its ramifications for tissue homeostasis, co-culture studies of organoids together with IELs and microbiota components would be useful. Several co-culture studies of immune cells with epithelial organoids have been established (FIG. 2; TABLE 2) and hold great promise for unravelling the bidirectional interactions between immune cells and epithelial cells. However, to obtain a more complete picture of how mucosal immunology is shaped, it will be important to introduce the effects of the microbiome to these co-cultures.

As has been reviewed elsewhere[97] and is discussed in the next section, organoids can be used to model host–pathogen interactions in response to bacteria[98,99], viruses[18,34,100,101] and parasites[102]. These co-culture studies have shown that the survival and proliferation of microorganisms can be modelled in organoids[103]. Although such studies have focused on pathological settings, organoids could also allow direct interactions between the immune system and the epithelium to be assessed in the presence of a commensal microbiota in settings that model intestinal homeostasis. Microorganism-derived metabolites (mainly short-chain fatty acids (SCFAs)) greatly affect mouse intestinal organoids in terms of gene transcription, in a microbial species-specific manner[104], as has been shown using conditioned media from microbial cultures. There is strong evidence that these microbial metabolites also affect the intestinal immune system[105]. Through their immunomodulatory effects, SCFAs may protect from mucosal inflammation[106–108] and tumorigenesis[109,110], as well as having systemic effects[111–114]. However, it is unclear what role the epithelium itself has in shaping the immune response to microbial metabolites. Further insights into this complex interaction between microbial metabolites, the immune system and the epithelium could be gained from combining these three elements in co-culture studies.

Studying infection and tissue repair

One of the main functions of the immune system is to contain infections upon tissue damage and then prevent such infections from spreading through the body. Upon clearance of the infection, the immune system also has a central role in tissue repair. As is the case for homeostatic interactions, epithelial organoids provide a good platform for modelling and studying immune cell–epithelial cell interactions in the context of pathogenic infections or sterile tissue damage.

Pathogenic infection. Several groups have studied the epithelial response to pathogenic infection using organoids. In these settings, organoids are typically used as a tool to culture microorganisms in mammalian cells. For example, *Helicobacter pylori*, which is a Gram-negative bacterium and the causative agent of gastric ulcers[115], has been microinjected into human iPSC-derived gastric organoids, where it tightly associates with the epithelium and subsequently transfers the virulence factor cytotoxin-associated gene A (CAGA) into the epithelial cells[116]. This triggers phosphorylation of the hepatocyte growth factor receptor MET and epithelial cell proliferation, as is observed during in vivo infection[117]. *H. pylori* induces expression of the checkpoint inhibitor programmed cell death 1 ligand 1 (PDL1) on human iPSC-derived gastric organoids[116], which could potentially lead to immune escape. Indeed, co-culture with autologous cytotoxic T lymphocytes (CTLs) did not have any effect on organoids in the absence of programmed cell death 1 (PD1)-blocking antibodies[116]. This exemplifies that organoids can be used not only as a tool for culturing pathogens that are otherwise difficult to culture but also as a model for antigen recognition and immune regulation.

In another organoid system, *Clostridium difficile* was microinjected into human iPSC-derived intestinal organoids[99]. Interestingly, whereas a virulent *C. difficile* strain disrupted epithelial integrity in the organoid, this was not seen for a non-virulent strain, which again underscores that epithelial organoids are a good model system for host–pathogen interactions[99]. Mechanistically, the major virulence factor of *C. difficile*, toxin B (TCDB)[118], binds to frizzled (FZD) proteins in intestinal crypts[118], leading to damage to Paneth cells and culminating in reduced regenerative capacity in the intestine[119]. Many cell types are involved in the inflammatory response to bacterial infection, which makes it difficult to dissect the exact mechanisms responsible for tissue damage and repair. Using mouse and human organoids, it was shown that constitutive STAT5 signalling rescued epithelial damage by inducing the differentiation of Paneth cells from ISCs[83]. Overactivation of STAT5 not only rescued organoids upon TCDB treatment but also induced tissue repair after ionizing radiation or tissue damage induced by pro-inflammatory cytokines[83]. The latter data show that the described mechanism is more broadly applicable than to antimicrobial responses alone.

The protozoan parasite *Cryptosporidium* is a major cause of diarrhoea and a cause of mortality in immunodeficient individuals, such as those with HIV/AIDS, elderly people and malnourished children[98,117,120–122].

Upon infection in human intestinal organoids, *Cryptosporidium parvum* could complete its life cycle in vitro, which was not the case in previously used models[102]. Furthermore, the transcription of type I interferon-related genes by IECs was increased, as would be expected following infection with parasitic pathogens[99].

Several reports have shown that organoids support the replication of viruses that are known to be tropic to

Table 2 | **Overview of the currently established organoid–immune cell co-culture systems**

Tissue of origin for organoid	Immune cell type	Medium used	Readout	Refs
Studying immune-mediated epithelial cell differentiation				
Mouse small intestine	Lamina propria lymphocytes and ILC3s	Organoid medium supplemented with recombinant mouse IL-2, IL-7, IL-15 and IL-23, or recombinant mouse IL-23 alone	IL-22-dependent stem cell proliferation	80
Mouse small intestine	Pre-stimulated $CD4^+$ splenocytes	Organoid medium	Intestinal stem cell differentiation	60
Human fetal intestine	Pre-stimulated fetal lamina propria T cells	Organoid medium without p38 MAPK inhibitor, supplemented with recombinant human IL-2	Organoid outgrowth	39
Studying epithelium-mediated immune cell differentiation				
Mouse small intestine	αβ and γδ IELs	Organoid medium supplemented with recombinant mouse IL-2, IL-7 and IL-15	IEL survival, proliferation and incorporation in epithelium	92
Mouse small intestine	OT-I $CD8^+$ splenocytes	T cell medium supplemented with EGF, noggin and R-spondin 1 or R-spondin 3	T cell proliferation and IEL phenotype	93
Mouse small intestine ($LGR5^+$ stem cells at single-cell level)	OT-II splenocytes (naive)	Organoid medium	T cell proliferation	60
Studying host–pathogen interactions				
RSV-infected human airway	Neutrophils	Airway organoid medium	Neutrophil chemoattraction	18
Helicobacter pylori-infected human gastric tissue	Peripheral blood T cells and DCs (autologous)	Organoid medium	T cell activation and proliferation, and organoid cytolysis	116
Studying tumour immunology				
Human breast cancer (single cells)	Peripheral blood and tumour-derived γδ T cells	T cell medium	Bisphosphonate-dependent γδ T cell activation and tumour cell line cytolysis	142
Air–liquid interphase culture of CRC or NSCLC	$CD45^+$ tumour-resident leukocytes	Organoid medium (including WNT3A)	In vitro survival of $CD45^+$ cells	143
MDOTS or PDOTS (derived from Merkel cell carcinoma or melanoma)	Tumour-derived leukocytes	MDOT or PDOT medium	Immune cell retention and tumour cytolysis upon introduction of immune checkpoint inhibitors	144
MDOTS (derived from mouse CRC cell line)	Tumour-derived leukocytes	MDOT medium	T cell activation and tumour cytolysis upon introduction of CDK4 and CDK6 inhibitors plus immune checkpoint inhibitors	145
Air–liquid interphase organoids (derived from human and mouse CRC, and kidney, lung or pancreas tumours)	Tumour-derived leukocytes	Organoid medium (including WNT3A)	Immune cell and TCR repertoire retention, and T cell activation and organoid cytolysis, upon introduction of immune checkpoint inhibitors	146
Mouse gastric cancer	$CD8^+$ splenocytes and bone marrow-derived DCs	Organoid medium	Organoid cytolysis	147,148
Human CRC	Peripheral blood $CD8^+$ T cells (autologous)	T cell medium supplemented with recombinant human IL-2 and PD1-blocking antibody	T cell activation and proliferation and organoid cytolysis	161
Human CRC	$CD8^+$ tumour-infiltrating lymphocytes	CRC medium supplemented with recombinant human IL-2 and PD1-blocking antibody	Organoid cytolysis	162
Human CRC	CAR T cells	Organoid medium without nicotinamide	CAR-dependent organoid cytolysis	165

CAR, chimeric antigen receptor; CDK, cyclin-dependent kinase; CRC, colorectal cancer; DC, dendritic cell; EGF, epidermal growth factor; IEL, intraepithelial lymphocyte; ILC3, group 3 innate lymphoid cell; MAPK, mitogen-activated protein kinase; MDOTS, mouse-derived organotypic tumour spheroid; NSCLC, non-small-cell lung cancer; PD1, programmed cell death 1; PDOTS, patient-derived organotypic tumour spheroid; RSV, respiratory syncytial virus; TCR, T cell receptor; WNT3A, WNT family member 3A.

the cultured organ. For example, herpes simplex virus and human papilloma virus readily infect and replicate in organoids derived from human oral mucosa[123], and human norovirus readily replicates in monolayers cultured from human small intestinal organoids[34]. Also, respiratory syncytial virus (RSV) replicates in human airway organoids[18], iPSC-derived organoids[101] and airway organoids cultured under air–liquid interphase conditions[100]. RSV infection caused large-scale epithelial remodelling, transcription of pro-inflammatory genes and the secretion of pro-inflammatory cytokines[18,100], as well as the recruitment of co-cultured neutrophils to infected organoids[18].

In summary, insights gained from such studies can enhance our understanding of the direct effects of pathogens on the epithelium. As the epithelium itself greatly influences the immune system by producing immunomodulatory factors, these data can then be used to infer the downstream effects of the epithelium on the immune system. Organoid–pathogen–immune cell co-culture studies could then be used to confirm these findings in a robust model system.

Tissue repair and regeneration. The immune system is also involved in tissue repair following epithelial injury[124]. For example, ILC3-derived IL-22 protects the intestine from damage caused by radiation, chemotherapy or graft-versus-host disease[54,80,125]. As we described above, IL-22 induces the survival and proliferation of LGR5$^+$ ISCs independently of other factors and independently of the presence of Paneth cells[80]. Intriguingly, intestinal recovery from irradiation, parasitic infection or general T cell overactivation resulted in the appearance of LY6A$^+$LGR5$^-$ cells in the bottom of intestinal crypts[126]. These LY6A$^+$ cells grew out into cystic organoids that resembled fetal intestine-derived spheroids[126,127], which suggests that recovery from tissue injury induces a fetal-like developmental programme in ISCs that depends on pro-inflammatory factors[126]. Inflammatory cytokines induce tissue repair also in other organs. For example, the pro-inflammatory cytokine TNF, but not IL-6, was found to promote long-term culture of primary mouse hepatocyte organoids in an NF-κB-dependent manner[11]. Importantly, both mouse and human organoid lines could be grown without TNF[9], which shows that although low-grade inflammation is necessary for epithelial regeneration, it is not required under steady-state conditions.

The complement system is a complex network of proteins that, once triggered, culminates in the release of anaphylatoxins and the formation of attack complexes on the membrane of target cells[128]. C3, a central player in the complement cascade that is cleaved into C3a and C3b upon activation, has been shown to be produced by WNT protein-dependent mouse ISCs[129]. Organoids from C3-deficient mice did not grow as efficiently as organoids from wild-type mice, and C3a supplementation rescued the growth of *C3a*$^{-/-}$ organoids[129]. The addition of C3a also rescued organoid growth upon blockade of WNT signalling, which suggests that C3a mediates its effects in the intestine at least partially by reinforcing WNT signalling[129]. Because C3 production was increased in mice after ischaemia–reperfusion injury, this suggests that complement has a role in intestinal regeneration upon injury in vivo[129]. In line with this, deleterious effects on liver and retinal regeneration following injury were found in mice deficient for complement factors C3 and C5 (REFS[130–132]). It was assumed in these studies that this effect was at least partially mediated by haematopoietic cells[131]. However, the organoid experiments provide proof that, at least in the intestine, the complement cascade can have direct effects on the epithelium[129]. In a similar manner, intestinal organoids grown from caspase 8-deficient individuals with very early onset IBD had an impaired apoptosis response when stimulated with the apoptosis-inducing cytokines TNF or TNF-related apoptosis-inducing ligand (TRAIL; also known as TNFSF10)[28]. This lack of an apoptotic response contributes to the disease phenotype. Importantly, these patients also have multiple defects in their haematopoietic compartment[28]. The use of organoids to study these patients has provided evidence that their phenotype is not only dependent on haematopoietic cells, which provides an explanation for their lack of response to immunosuppressive therapy.

In summary, the presence of both stem cells and differentiated cell types in organoids enables their use to provide an accurate assessment of the behaviour of healthy epithelium both under steady-state conditions and upon tissue injury. The experiments described were invaluable in uncoupling the role of the epithelium from the role of the immune system in the initiation and maintenance of inflammatory responses.

Studying the tumour microenvironment

Tumour-initiating cells typically 'highjack' the signalling pathways that regulate adult stem cell function through the acquisition of mutations that overcome normal cell cycle control mechanisms[26,133]. Hence, established organoid protocols could be adapted to grow epithelial cancer organoids from patient-derived tumour tissue[27,134]. Such organoid cultures have been established from primary cancer tissue of the colon and rectum[135–137], liver[138] and lung[18], among other organs[27]. The tumour microenvironment contains various non-epithelial cell types, including immune cells and stromal cells, which greatly influence therapeutic responses[139]. However, modelling this tumour microenvironment in vitro has been a major challenge in the past[139]. In particular, cancer cell lines and patient-derived xenograft models have limitations to their uses[139]; the former are insufficient to recapitulate the heterogeneity of tumour epithelial cells[140], and the latter typically depend on the mouse immune system, which does not adequately replicate the human immune system[139]. In light of promising therapeutic developments that use a patient's own immune system to eradicate tumour cells, in the form of cancer immunotherapy[141], several organoid-based models have emerged to better study these responses. Two conceptually different approaches have been described so far: the holistic approach uses cancer organoids cultured directly from tumours while preserving endogenous immune cells (and other non-epithelial cell types), whereas a more reductionist approach uses cancer organoids co-cultured

Air–liquid interphase
A culture method in which cells are grown in a monolayer on a transwell, allowing for contact both with air and with a culture medium in the bottom well.

Graft-versus-host disease
A condition occurring upon allogeneic bone marrow transplantation in which graft-derived immune cells mount an immune response against the host tissues.

Patient-derived xenograft
A system in which pieces of patient-derived tumour material (including the entire tumour microenvironment — in other words, immune cells, endothelial cells and fibroblasts, as well as the tumour epithelium) are transplanted into immunocompromised mice, which allows for the study of tumour behaviour in an in vivo system.

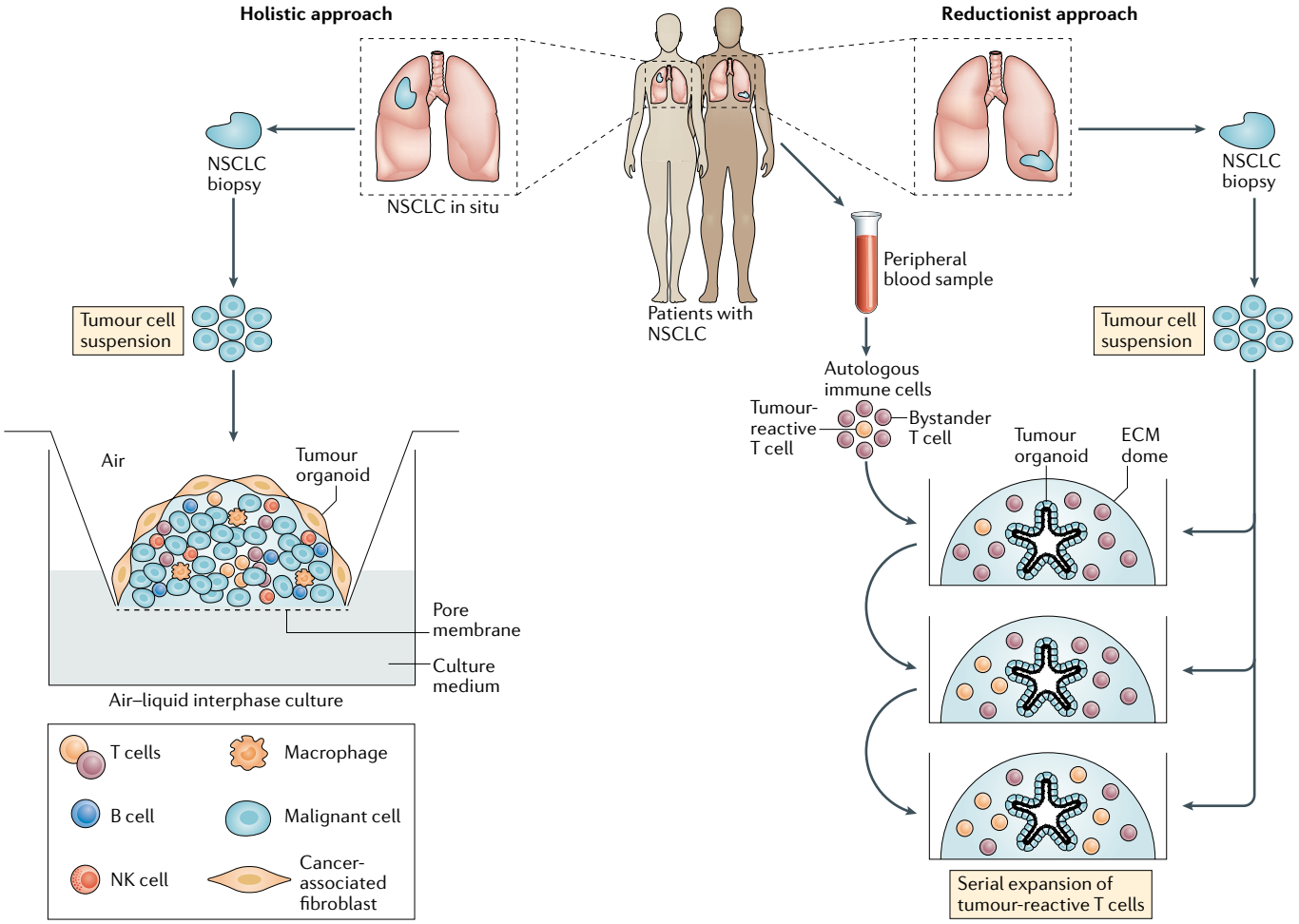


Fig. 3 | **Organoid–immune cell co-culture systems in immuno-oncology research.** Two main approaches are currently being used, shown here using the example of patients with non-small-cell lung cancer (NSCLC). In the holistic approach (left), tumour biopsies are cultured in air–liquid interphase as a cell suspension of all tumour cell types, including endogenous immune cells and other non-epithelial cell types, which promotes the outgrowth of tumour-specific T cells (among others). In the reductionist approach (right), epithelial organoids are grown from tumour biopsies and are then co-cultured with autologous immune cells from the peripheral blood of the same patient, to promote the serial expansion of tumour-reactive cells. Whereas the holistic approach allows for the culture of tumour material that includes the entire tumour microenvironment, and thus closely resembles the in vivo situation, the reductionist approach allows for the long-term culture and expansion of the tumour epithelium, which enables more extended investigations. ECM, extracellular matrix; NK cell, natural killer cell.

with (isolated and separately expanded) immune cell subsets (FIG. 3).

Holistic approaches using cancer organoids. In a 2016 study, organoids from normal human epithelial breast tissue were generated that retained IELs[142]. The authors then exposed the organoid cultures for up to 4 weeks to aminobisphosphonate drugs that have been shown to stimulate a subset of IFNγ-producing T cells[142]. In a subsequent assay, these activated Vδ2$^+$ T cells efficiently killed breast cancer cells. Another study showed that haematopoietic cells could be maintained for up to 8 days in co-culture with human tumour organoids generated from different types of epithelial cancer cultured in air–liquid interphase conditions[143]. Although it was unclear which types of haematopoietic cell remained viable, the authors reported reduced T cell survival in their model system[143]. In early 2018, two studies described microfluidic-based methods to model anti-PD1 and anti-PDL1 cancer immunotherapy using short-term cultures of mouse-derived and patient-derived organotypic tumour spheroids (MDOTS and PDOTS, respectively)[144,145]. In the first study, MDOTS were generated by transplanting mouse cancer cell lines (for example, a melanoma-derived cell line) into immunocompetent mice. When cultured in microfluidic devices, these MDOTS were found to retain autologous haematopoietic cell populations that responded to PD1 blockade. The therapeutic response or resistance to checkpoint blockade in vivo was recapitulated in the MDOTS model. Subsequently, the authors established PDOTS from immunotherapy-responsive cancer types, such as melanomas. Anti-PD1 treatment of the PDOTS allowed for the identification of secreted cytokines and chemokines (such as CC-chemokine ligand 19 (CCL19) and CXC-chemokine ligand 13 (CXCL13)) that

Organotypic tumour spheroids
A culture system in which pieces of tumour (including the entire tumour microenvironment — in other words, immune cells, endothelial cells and fibroblasts, as well as the tumour epithelium) are brought into culture, which allows for the study of tumour characteristics in vitro.

may be associated with ineffective antitumour immunity[144]. In the second study, MDOTS generated from a mouse colorectal cancer (CRC) cell line were used to validate observations made in vivo that blockade of cyclin-dependent kinase 4 (CDK4) and CDK6 using selective small-molecule inhibitors promoted T cell activation and killing of tumour cells. MDOTS treated with an inhibitor of CDK4 and CDK6 plus PD1 blockade had increased levels of tumour cell death. MDOTS generated in immunocompromised mice and subjected to the same treatment regime did not have a similar antitumour response, which suggests that T cells were responsible for the synergistic effect of CDK4 and CDK6 inhibition combined with PD1 blockade[145].

A recent report described a sophisticated air–liquid interface method using patient-derived organoids of different cancer types, including colorectal and lung cancers, that allowed for the preservation of the tumour epithelium and its stromal microenvironment in vitro[146]. In addition to stromal fibroblasts, cellular immune components such as tumour-associated macrophages, CTLs, T_H cells, B cells, natural killer (NK) cells and natural killer T (NKT) cells were readily maintained for up to 30 days in the organoid cultures. The T cell receptor (TCR) heterogeneity of the T cells found in the original tumour was also preserved in these cultures. The authors used these organoids to model immune checkpoint blockade, which resulted in the proliferation and activation of tumour antigen-specific T cells and subsequent tumour killing[146].

Reductionist approaches using cancer organoids. Reductionist approaches that initially establish organoids separately from immune cells before co-culture enable investigations of specific cell–cell interactions. For example, one study established triple co-cultures of mouse gastric cancer organoids with DCs and CTLs[147]. Organoids were generated from gastric cancer tissue isolated from transgenic mice or from the normal gastric tissue of control mice[16,147]. In parallel, DCs and CTLs were isolated from the bone marrow or spleens of tumour-bearing or control mice and co-cultured for 2 days in conditioned medium derived from normal or cancer organoids[147,148]. Following this initial DC–CTL co-culture, normal gastric organoids and gastric cancer organoids were mixed with DCs and CTLs in the presence of PDL1 inhibitors, and organoid killing was analysed after 16 hours of co-culture[147,148]. The authors observed significant death of cancer cells when cancer organoids were co-cultured with conditioned medium-pulsed DCs and CTLs in combination with anti-PDL1 checkpoint blockade[147].

Effective T cell-mediated antitumour responses depend on tumour (neo-)antigens, which are themselves largely determined by the mutational load of tumour cells[149–156]. Therefore, in vitro T cell-mediated killing assays have classically been carried out with melanoma tumour cells as the target cells; melanomas typically have a high mutational burden and elicit robust CTL activation, which makes them ideal targets for cancer immunotherapy involving immune checkpoint blockade[155,157–159]. The use of epithelial cancer organoid cultures has allowed for the broadening of in vitro assays to other epithelial tumour types, as stable tumour cell cultures could also be derived from epithelial cancers with a high mutational burden, such as mismatch repair-deficient (MMR-deficient) CRCs[136,137,140,160] and subtypes of non-small-cell lung cancers (NSCLCs)[18]. Organoids from primary or metastatic CRC or NSCLC with high mutational burden were co-cultured with autologous peripheral blood mononuclear cells (PBMCs) in the presence of IL-2, PD1 blockade and plate-bound, agonistic CD28-specific antibody[161]. Here, cancer organoids function to provide antigen-specific stimulation of T cells in the PBMC fraction. CTLs that were expanded in the co-culture effectively killed cancer organoids. Healthy lung organoids generated from the same patients continued to proliferate in the presence of CTLs without evidence of T cell-mediated cytotoxicity[161]. This proof-of-concept study suggests that cancer organoids present antigens that can trigger T cell proliferation and stimulation. The authors did not assess TCR clonality in the tumour-reactive T cell sublines. MMR-deficient tumours, in particular, are characterized by a high degree of genetic diversification[140,160], which in turn creates a genetically heterogeneous pool of cancer cells. It would therefore be interesting to assess how genetic diversification of the clonal organoid cultures may result in antigen diversity that could elicit a heterogeneous antitumour response by T cell clones raised against different cancer organoid clones and antigens. As was discussed by the authors, their strategy to generate patient-specific tumour-reactive T cells only succeeded in ~50% of cases, in which tumours had high mutational loads and expressed MHC class I molecules.

Cancer organoid–T cell co-cultures may also help to predict the functionality of tumour-infiltrating lymphocytes (TILs) upon chemoradiotherapy and after immune checkpoint blockade. In a proof-of-principle study[162], CRC organoids and TILs were expanded separately and then co-cultured, and organoid killing by TILs was assessed using microscopy. Cytotoxic killing was significantly higher in co-cultures that were generated from patients with a complete response to chemoradiotherapy, compared with co-cultures established from non-responders[162]. In a second step, the authors applied an immune checkpoint blocking antibody against PD1 and showed that antitumour immunity could be partially restored in TILs with increased PD1 expression[162]. Therefore, such co-culture assays may be used as a platform to assess the efficacy of cancer immunotherapy in future.

Cancers with low mutational loads and stable (tumour) antigen presentation may be suitable targets for chimeric antigen receptor (CAR)-engineered T cells. Promising results in this respect have been obtained for B cell malignancies[163]. Currently, CAR T cell therapy is only used against leukaemia, primarily because no viable targets other than the B cell markers CD19 and CD20 have been described so far. The therapeutic use of CAR T cells in solid cancers such as CRC has been hampered by side effects that arise from targeting overexpressed native antigens that are, therefore, not exclusively expressed by tumours[164]. Recently, an assay has been developed to test CAR T cell-mediated cytotoxicity against cancer organoids[165]. Instead of

using CAR-engineered T cells, the authors used — as a proof of concept — an NK cell line with non-MHC-restricted cytolytic activity, in which CARs were introduced that target native peptides of human epidermal growth factor receptor 2 (HER2) and epithelial cell adhesion molecule (EPCAM), which are overexpressed by cancers. CAR-mediated cytotoxicity was observed against organoids derived from both normal colon tissue and CRC tissue presenting either peptide[165]. Subsequently, the authors engineered CARs targeting epidermal growth factor receptor variant III (EGFRvIII), a neoantigen that is widely expressed by solid cancers, including lung cancer[166]. In a competitive co-culture assay, EGFRvIII-specific CAR NK cells efficiently killed EGFRvIII-expressing cancer organoids but not normal-tissue organoids[165]. Finally, the team generated CARs targeting antigens specific to CRC subsets that overexpress the WNT ligand receptor FZD upon loss of expression of its antagonists ring finger protein 43 (RNF43) and/or zinc and ring finger 3 (ZNRF3)[136,167,168]. Targeting FZD proteins is a promising therapeutic approach, and neutralizing antibodies to FZD proteins have already been developed[169]. To test possible adverse effects of FZD-specific CARs, the authors evaluated cytotoxicity against normal colon organoids as well as different gene-edited organoid lines deficient for both RNF43 and ZNRF3 or for adenomatous polyposis coli (APC)[170] (which also overexpress FZD proteins). These co-culture assays showed that the cytolytic activity of the FZD-specific CAR NK cells was not specific to the mutant organoid lines, which suggests that such approaches may have marked side effects if used therapeutically[165].

In summary, with the establishment of organoids from various types of epithelial cancer, co-cultures of cancer organoids and immune cells have become a highly informative strategy for the development and testing of cancer immunotherapy.

Conclusions and future perspectives

Epithelial organoid cultures, whether derived from iPSCs or adult stem cells, provide a promising platform for immunological research for several applications. First and foremost, organoids allow for a reductionist investigation into the complex and close interactions between epithelial cells and immune cells. The effects of the immune system on epithelial differentiation and function have largely been studied in the intestine[60–62,70,71,76]. It will be of interest to see whether similar principles apply to other organoid systems, such as skin or lung, which also interact with both commensal microorganisms and immune cells. Epithelial composition changes upon infection and in response to cytokine triggers[60–62,70,71,76]; hence, potential bidirectional interactions between the immune system and epithelium, as are seen in the intestine, can be readily studied in vitro using organoids. Such studies will add to our understanding of the biology of complex inflammatory diseases, ranging from IBD to multiple sclerosis, psoriasis and asthma.

Organoids are also being used to study interactions between the host and microorganisms[18,97,102], and the next step in that direction would be to add immune system components to infected organoids. A limited number of approaches using such triple co-cultures have been developed, mostly mimicking pathogenic infections with viruses or bacteria[18,116]. Here, organoids have been infected with pathogens (viral or bacterial) before co-culture with immune cells. In recent years, it has been shown that the commensal flora has a pivotal role in safeguarding mammalian homeostasis[171,172], and it has been suggested that this is at least partly based on interactions with the epithelium. Epithelial organoid technology provides a unique opportunity to investigate all three components (epithelium, immune system and commensal microorganisms) in a defined system. Interactions between these components have a crucial role in many human diseases, such as IBD and asthma. Obtaining a better understanding of these interactions and their consequences is thus of great interest. In the future, optimization of (triple) co-culture systems will be important to the study of these interactions in both homeostatic and pathological conditions, possibly leading to new therapeutic approaches.

Finally, tumour-derived organoids, which represent the transcriptional and mutational profile of the original tumour[27], provide a new and reliable model system for the interaction of the immune system with tumour cells. Several tumour-derived organoid systems have been shown to faithfully recapitulate the response of the tumour to various therapies, including chemotherapeutics and radiation[27]. This faithful recapitulation of tissue responses in vitro is also interesting in other pathological conditions and has been exemplified in the case of cystic fibrosis, in which patient-derived organoids can be used to predict a patient's response to therapy[173,174]. It is appealing to use organoids as a platform for developing and testing personalized medicine approaches, as they are in essence cultures of primary patient material. While this is now possible for cystic fibrosis and cancer[123,136,173,175], it could also be possible for inflammatory disorders that affect epithelial tissues. To this end, organoid-based bioassays need to be standardized and brought to clinical grades, and diagnostic tests including organoids should be incorporated into clinical trials.

As cancer immunotherapy is taking a more central role in the clinic, several reports have shown that tumour-derived organoids can be used to model the effects of such novel therapies[148,161,165]. However, although tumour-derived organoids show promise for future research, some important limitations should be considered. Primarily, organoids are often derived from biopsies, which represent only a small part of the tumour tissue. Therefore, the complexity of the whole tumour will always be underestimated by the organoid. Also, tumour-derived organoids are not exposed to the external pressures that occur in situ, such as hypoxia or immune selection. Selection can influence the outgrowth of tumour clones, leading to a situation in which a dominant clone in vitro is not as dominant in situ, and vice versa. Importantly, these limitations become more significant when considering hypermutated tumours, such as MMR-deficient CRC or NSCLC[140,160].

More generally, the co-culture conditions for organoids are often a compromise between the optimum conditions for each of the included cell types. As culture conditions can influence cellular behaviour in many ways, it is important to keep this in mind when considering long-term co-culture experiments. To this end, one focus of investigators in the coming years will be the further optimization of co-culture conditions, keeping in mind factors such as media composition and the type of ECM used. Having noted these limitations, organoids currently provide the most accurate in vitro system for the culture of human epithelial cells of almost any organ and show great promise for both fundamental and translational research in the future.

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Y.E.B.-E. and K.K. researched data for the article and wrote the manuscript. All authors contributed equally to discussion of the content and to reviewing and editing the manuscript before submission.

Competing interests
The authors are inventors on patent applications and/or patents for organoid culture and organoid–immune cell co-cultures.

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