Microbiota in pancreatic health and disease: the next frontier in microbiome research

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Abstract | Diseases intrinsic to the pancreas such as pancreatitis, pancreatic cancer and type 1 diabetes mellitus impart substantial health and financial burdens on society but identification of novel mechanisms contributing to these pathologies are slow to emerge. A novel area of research suggests that pancreatic-specific disorders might be modulated by the gut microbiota, either through a local (direct pancreatic influence) or in a remote (nonpancreatic) fashion. In this Perspectives, we examine literature implicating microorganisms in diseases of the pancreas, specifically pancreatitis, type 1 diabetes mellitus and pancreatic ductal adenocarcinoma. We also discuss evidence of an inherent pancreatic microbiota and the influence of the intestinal microbiota as it relates to disease association and development. In doing so, we address pitfalls in the current literature and areas of investigation that are needed to advance a developing field of research that has clinical potential to reduce the societal burden of pancreatic diseases.

The field of microbiota research has rapidly evolved from the initial focus on enumerating microorganisms (bacteria, viruses and fungi) present in various human body locations to experiments elucidating mechanisms by which the microbiota influences health and disease conditions. Intrinsic diseases of the pancreas, such as type 1 diabetes mellitus (T1DM), pancreatitis and pancreatic ductal adenocarcinoma (PDAC) affect a large proportion of the population[1–3], result in marked morbidity and mortality[3–5] and contribute to a substantial portion of health-care costs annually[6–8]. For example, pancreatitis was responsible for over 757,000 office or emergency room visits combined in the USA in 2014, with an aggregate cost of US$2.77 billion[8]. Despite the high prevalence of pancreatic disease and the homeostatic role that the pancreas has in gastrointestinal physiology, and its anatomical relationship to the gastrointestinal tract, investigations regarding the role of the microbiota in pancreatic health and disease are limited. Additionally, new therapies, risk identification and improvement in pancreatic disease treatment and survival have been relatively stagnant compared with other gastrointestinal diseases. Accordingly, novel investigations are necessary to improve human health related to diseases intrinsic to the pancreas.

Studies are beginning to support the existence of interactions between various host microbiomes and the pancreas that include immune regulation, two-way communication in which pancreas-derived antimicrobial peptides can influence the gastrointestinal microbiota, and the effect of the microbiome on chemotherapeutic regimens used to treat PDAC[9–13]. These studies have opened new research avenues for both benign and malignant pancreatic diseases. In this Perspectives, we discuss evidence supporting a microbiome–pancreas axis for diseases originating in the pancreas with a focus on PDAC. These studies will be put into perspective regarding gaps in our current knowledge and how this line of research can be advanced. To focus on diseases intrinsic to the pancreas, and because alterations in glucose homeostasis as a result of type 2 diabetes mellitus are secondary to peripheral insulin resistance[14], the role of the gut microbiota in obesity and metabolic syndrome that contributes to insulin resistance and type 2 diabetes mellitus is not addressed here and we refer the reader elsewhere for in-depth reviews regarding this topic[15–17].

Microbiota and the pancreas

Gut microbiota in normal pancreas physiology. The physiological importance of bacteria within the intestine has been recognized through their effects on immune regulation, pathogen niche exclusion and nutrition[18–20]. However, none of these functions has been ascribed to the pancreas. Given that the pancreas is anatomically connected to the gastrointestinal tract via the pancreatic duct and communicates with the liver via the common bile duct (CBD), this intimate relationship of the pancreas to the gastrointestinal tract leads to the question of whether the intestinal microbiota, or even an intrinsic pancreatic microbiota, might impart similar homeostatic properties to this organ, as it does to the intestines. Indeed, investigations of microbial composition have extended to the pancreas (TABLE 1). Once thought to be a sterile organ, a number of studies have now established the presence of a microbiota within this organ in normal, nonpathological states, albeit with discordant results between studies regarding the microbial composition[9,10,21]. As opposed to the existing controversy around the placenta microbiome, the presence of bacteria in the pancreas has not been challenged[22,23]. These studies have been stringently conducted to account for contamination by using either DNA extraction kit controls, blank controls, in situ detection or culture of bacteria. The studies all converged to the same conclusion that microorganisms inhabit the pancreas. Several groups independently analysed the microbiome of normal human pancreas via 16S ribosomal RNA (rRNA) gene sequencing. Notably, the definition of 'normal' varied between groups or was not clearly defined. Specifically, the normal pancreas consisted of specimens from pancreata resected for nonmalignant aetiologies (for example, benign cysts), the nonmalignant surgical margin acquired from a malignant surgical specimen or from organ donors[9,10,13]. In a study by Pushalkar et al.[10], the increased relative

Table 1 | **Summary of major microbiome studies involving pancreatic disease**

Author	Study population	Disease states versus control[a]	Microbiome specimen	Microbiome evaluation	Microbial change
Jandhyala[57]	Human	CP vs normal	Faeces	16S sequencing	↑ Firmicutes ↓ Bacteroidetes
Isaiah[62]	Canine	EPI vs normal	Faeces	16S sequencing	↑ Lactobacillaceae and Streptococcaceae ↓ Lachnospiraceae and Ruminococcaceae
Hamada[61]	Human	CP vs AIP	Faeces	16S sequencing	↑ *Bacteroides*, *Streptococcus* and *Clostridium* spp.
Zhang[55]	Human	AP vs normal	Faeces	16S sequencing	↑ Bacteroidetes and Proteobacteria ↓ Firmicutes and Actinobacteria
Beger[44]	Human	AP	Pancreas	Culture	NA
Büchler[45]	Human	AP	Pancreas	Culture	NA
Isenmann[46]	Human	AP	Pancreas	Culture	NA
Thomas[9]	Human	CP vs normal	Pancreas	16S sequencing	NA
Wen[80]	Mouse	DM	Faeces (reconstitution)	NA	NA
Kostic[138]	Human	DM	Faeces	16S sequencing	NA
de Goffau[71]	Human	DM vs normal	Faeces	16S sequencing	↑ *Bacteroides* ↓ *Bifidobacterium adolescentis* and *Bifidobacterium pseudocatenulatum*
Endesfelder[139]	Human	DM vs normal	Faeces	16S sequencing	NA
Mejía-León[73]	Human	DM vs normal	Faeces	16S sequencing	↑ *Bacteroides* ↓ *Prevotella*, *Megamonas* and *Acidaminococcus*
Davis-Richardson[74]	Human	DM vs normal	Faeces	16S sequencing	↑ *Bacteroides dorei* and *Bacteroides vulgatus*
Alkanani[140]	Human	DM vs normal	Faeces	16S sequencing	↓ *Lactobacillus* and *Staphylococcus*
Vatanen[72]	Human	DM vs normal	Faeces	16S sequencing	NA
Hu[141]	Mouse	Pre-DM vs normal	Faeces	16S sequencing	↑ Gram-negative and Gram-positive ↓ *Bacteroidetes* and Erysipelotrichaceae
Farrell[30]	Human	PDAC vs normal	Oral	16S microarray	↓ *Neisseria elongata* and *Streptococcus mitis*
Fan[29]	Human	PDAC vs normal	Oral	16S sequencing	NA
Michaud[88]	Human	PDAC vs normal	Plasma	Antibiotics to oral bacteria	↑ *Porphyromonas gingivalis* (ATTC 53978)
Ren[89]	Human	PDAC vs normal	Faeces	16S sequencing	↑ Bacteroidetes ↓ Firmicutes and Proteobacteria
Pushalkar[10]	Human	PDAC vs normal	Faeces	16S sequencing	↑ Proteobacteria, Synergistetes and Euryarchaeota
		PDAC vs normal	Pancreas	qPCR	NA
Thomas[9]	Human	PDAC vs normal	Pancreas	16S sequencing	NA
Riquelme[110]	Human	PDAC LTS vs PDAC STS	Pancreas	16S sequencing	↑ Alpha diversity; ↑ *Saccharopolyspora*, *Pseudoxanthomona*, *Streptomyces*

AIP, autoimmune pancreatitis; AP, acute pancreatitis; CP, chronic pancreatitis; DM, diabetes mellitus; EPI, exocrine pancreatic insufficiency; LTS, long-term survivor; NA, not applicable; PDAC, pancreatic ductal adenocarcinoma; qPCR, quantitative PCR; STS, short-term survivor. [a]Normal control or reference specimens varied between studies and represent cadaveric specimens, surgical margins of resected pancreas in which pathology such as pancreatic cancer was not identified, and/or healthy volunteers (faecal, oral specimens).

abundance of the genus *Brevibacterium* and the order Chlamydiales was seen in normal pancreata compared with patients with PDAC. However, Thomas and colleagues noted increased *Acinetobacter* and *Pseudomonas* in their normal specimens compared with PDAC, but the differences were not statistically significant after false discovery rate correction[9]. Finally, studies have also detected bacteria within healthy control pancreas specimens by either 16S rDNA quantitative PCR (qPCR) or culture techniques in cadaveric pancreata[13].

The presence of a microbiota in normal pancreata calls into question its role in pancreatic physiology. Antimicrobial peptides (AMPs) are secretory components involved in antibacterial innate immunity in the gastrointestinal tract. They are primarily secreted by intestinal Paneth cells directly into the intestinal lumen but other organs, including the pancreas, also contribute to this innate defence mechanism[24]. The AMPs of the pancreas contribute ~10% of the proteins found in pancreatic juice, with the remaining represented primarily by digestive enzymes[25,26]. Multiple pancreatic

AMPs have been identified as being produced by acinar cells (defensin α1), islet cells (defensin β3) or both (defensins β2 and α4, cathelicidin-related antimicrobial peptide (CRAMP))[11,12,27]. A bidirectional crosstalk is present whereby pancreatic AMPs are influenced by the gut microbiota to modulate intrapancreatic immune cells as well as the secretion of AMPs into the gastrointestinal tract via pancreatic fluid, which subsequently alter the gut microbiome and the intestinal immune system[11,12]. For example, CRAMP production by pancreatic β-cells has been shown to be controlled by short-chain fatty acid (SCFA) production by the gut microbiota[11]. This CRAMP production, in return, results in a phenotypic switch of intrapancreatic macrophages from an inflammatory to a regulatory phenotype, accomplished via a decrease in macrophage TNF production and an increase in transforming growth factor-β production. Additionally, CRAMP production induces conventional dendritic cells as well as regulatory T cells within the pancreas, creating a immunoregulatory environment[11]. In a separate set of studies, the effect of AMPs on the gut microbiome and gut innate immunity was elucidated[12]. In C57BL/6 mice, deletion of *Orai1*, a store-operated Ca^{2+} channel involved in cellular membrane pore formation during pancreatic acinar cell exocytosis for pancreatic juice secretion[28], resulted in unexpected animal death, which was secondary to bacterial overgrowth. This bacterial overgrowth shifted the gastrointestinal microbiota to a pro-inflammatory phenotype, which was secondary to decreased AMP (CRAMP) secretion[12] (FIG. 1). Whether the AMPs modulate the pancreatic microbiota is unknown but might ultimately serve to influence immune homeostasis with cues taken from AMP production.

On the basis of accumulating data, the pancreas likely possesses a microbiota, but a caveat should be made given the variable definitions of 'normal' and the fact that germ-free mice transferred to specific pathogen-free (SPF) conditions do not acquire pancreatic bacteria up to 8 weeks after transfer despite oral gavage of bacteria[9]. Additionally, a paucity of bacteria have been noted by 16S qPCR in normal mice pancreata compared with *Kras*-mutant or *Trp53*-mutant mice[10]. The ultimate role of these intrapancreatic bacteria remains to be elucidated, especially in light of the inherent antimicrobial activity present within pancreatic juice.

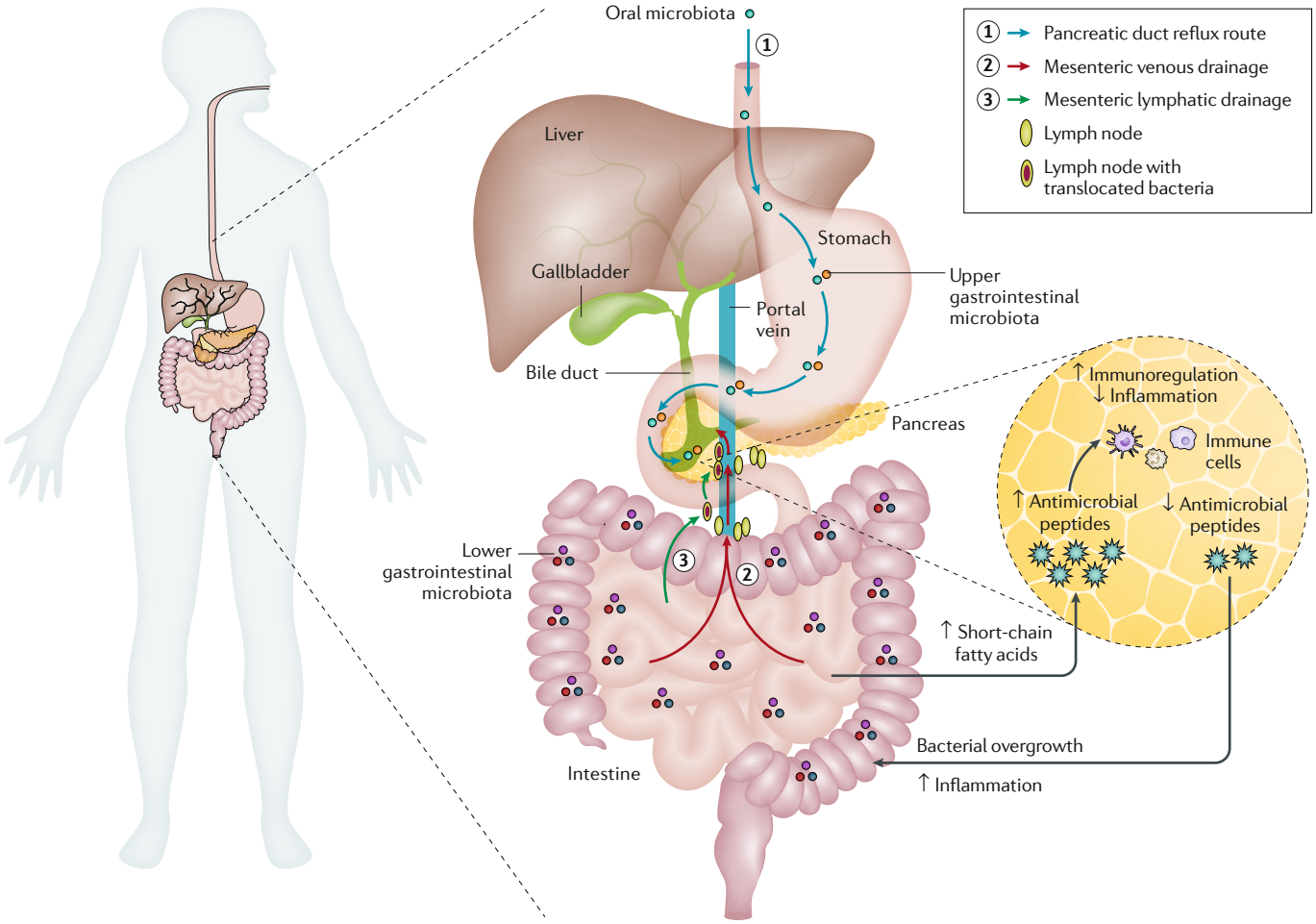

Fig. 1 | **Proposed models of bacterial translocation to the pancreas and the homeostatic host response.** The presence of a pancreatic microbiota has been shown to occur in a variety of normal and diseased states. The routes whereby bacteria can gain access to the pancreas are debated, but several mechanisms such as the oral route (1), via translocation from the lower gastrointestinal tract through the portal circulation (2) or mesenteric lymph nodes (3) are supported by the literature and are illustrated. Additionally, pancreatic antimicrobial peptides (AMPs) can have homeostatic bidirectional communication with the gastrointestinal tract, whereby the lower gastrointestinal microbiota influences pancreatic AMP production through short-chain fatty acid metabolites to induce an immunoregulatory pancreatic environment with decreased pro-inflammatory immune cells. Conversely, decreased AMP production by the pancreas enables gastrointestinal microbiota overgrowth and development of a pro-inflammatory phenotype.

Proposed bacterial colonization of the pancreas. How pancreatic microbiota might be established is an area of debate, as several mechanisms have been proposed or can be envisioned. One such mechanism takes the anatomical relationship of the pancreas to the gastrointestinal tract into account. Its proximity to the upper gastrointestinal tract creates a plausible scenario whereby the microbiota of the oesophagus, stomach, duodenum or biliary tract can gain access to the pancreatic parenchyma via the pancreatic duct. For example, in the *Ptf1a*-Cre;*Kras*$^{LSL\text{-}G12D}$ mouse model of PDAC, the presence of *Bifidobacterium pseudolongum* in the pancreas following oral gavage of this bacterium (10^8 colony forming units) every other day for 2 weeks might be due to a reflux phenomenon of bacteria into the pancreatic duct[10]. Although this study might seem contradictory to that of Thomas et al.[9], this inoculation procedure with an extremely high bacterial load gavaged twice daily does not resemble natural colonization. Additionally, the mouse cohort that demonstrated intrapancreatic bacteria were the *Ptf1a*-Cre;*Kras*$^{LSL\text{-}G12D}$ mice, whereas the wild-type mice did not exhibit intrapancreatic bacteria after gavage, similar to that of Thomas et al. It is, therefore, unclear how bacteria naturally access the pancreas as other routes might also explain their intrapancreatic presence (FIG. 1).

Interestingly, microorganisms contributing to periodontal disease and associated with pancreatic cancer, such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Granulicatella adiacens*[29,30], have not routinely been found in pancreatic tissue by 16S rDNA sequencing[9,10]. Although a report in 2019 did identify bacterial taxa commonly found in the oral microbiota (*Fusobacterium*, *Prevotella*, *Dialister*, *Veillonella*, and *Haemophilus*) to be also present in the pancreas of patients with PDAC, as well as cadaveric healthy controls, by 16S rDNA sequencing, their conclusions were not corroborated with parallel oral microbiota sequencing[31]. These bacteria might merely be associated with factors such as tobacco use that alter the oral microbiota and are involved in pancreatic carcinogenesis.

Given the abundance of bacteria in the intestine, an alternative mode of bacterial seeding into the pancreas might occur through translocation from the gut. Anatomically, this explanation is feasible given that mesenteric venous drainage passes by the pancreas en route to the liver. Indeed, bacterial translocation from the intestine to mesenteric lymph nodes has been reported both autonomously as well as via immune cell trafficking[32,33]. Immune cell trafficking of normally noninvasive bacteria has been shown to occur in a CCR7-dependent manner by CX_3CR1^{hi} mononuclear phagocytes, a subtype of resident intestinal mononuclear phagocytes[34], during a state of antibiotic-induced dysbiosis or in the absence of *Myd88* (REF.[32]). This finding demonstrates the importance of commensal bacteria in regulating intestinal immunosurveillance through the trafficking of CX_3CR1^{hi} mononuclear phagocytes to mesenteric lymph nodes with captured bacteria from the lumen of the intestine[32]. This process, in which bacteria are 'screened' and trafficked from the intestine to mesenteric lymph nodes, might provide the opportunity for bacteria to gain access to the pancreas simply by anatomic lymphatic drainage routes, but details of this trafficking pattern are currently unknown (FIG. 1). Furthermore, even when intestinal permeability is compromised in *Il10*$^{-/-}$ mice following *Campylobacter jejuni* infection, no culturable or PCR detectable bacteria were observed in the pancreas of these *Il10*$^{-/-}$ mice[9]. However, bacterial infection of the pancreas has been shown to occur haematogenously via transmural translocation from the colon, or via reflux into the pancreatic duct when local inflammation within the pancreas (pancreatitis) is present[35]. Taken together, these findings suggest that bacterial acquisition is not a normal physiological process for the pancreas with or without intestinal inflammation, but might be a sign of underlying pathology within the pancreas that enables translocating bacteria to establish a niche.

Microbiota in pancreatitis. Acute pancreatitis is diagnosed by at least two of the following criteria: abdominal pain, serum amylase and/or lipase over three times the upper limit of normal of the laboratory conducting the analysis, and findings most commonly diagnosed by computed tomography with intravenous contrast[36]. Imaging findings during the acute process are subdivided into interstitial oedematous pancreatitis, characterized by local fat inflammation but enhancement of the pancreas on contrasted imaging, and necrotizing pancreatitis, characterized by a lack of enhancement of the pancreas on contrasted imaging which signifies death of the organ in that region[36]. Chronic pancreatitis is the long-term or recurrent process of inflammation with concurrent sequelae of an acute episode[36]. Several theories exist regarding the aetiology of pancreatitis and are outside the scope of this Perspectives but which have been covered elsewhere[37]. Although genetic predispositions exist that relate to mutations of the cationic trypsinogen gene (*PRSS1*), serine peptidase inhibitor, Kazal type 1 gene (*SPINK1*), or cystic fibrosis transmembrane conductance regulator (*CFTR*) mutations, this understanding only accounts for ~2% of all cases of chronic pancreatitis[38]. The major aetiology of pancreatitis, which is secondary to cholelithiasis or ethanol ingestion, represents a substantial health-care burden but is variable in morbidity and mortality[4,5,8,39]. Individuals with a high intake of ethanol might never develop sequelae of its ingestion, and even those who develop alcohol-induced hepatitis might never develop pancreatitis[40,41]. How these differences can be reconciled is an active area of investigation.

Although many cases of pancreatitis resolve without long-term morbidity, acute pancreatitis with or without necrosis results in substantial hospitalization, health-care costs and long-term disability for many people worldwide[2,5,8,42,43]. In pancreatic necrosis, ~30% of cases, although initially sterile, become infected, which has led to a debate regarding the use of prophylactic antibiotics in the setting of acute pancreatitis[44–46]. In such cases, studies have noted culture positivity primarily for *Staphylococcus*, *Enterococcus*, *Escherichia coli* and *Klebsiella* from the necrotic pancreatic tissue itself[44–46]. Such bacteria could have originated from the lower gastrointestinal tract, given that several are found in high abundance in this organ but not elsewhere in the body[47–49]. Although bacteria might not be the inciting reason for the pancreatitis, the inflammatory environment might enable entry of these microorganisms into the pancreas and worsen the local and systemic inflammatory condition, which would be congruent with theories that clinical sepsis originates from the gut[50,51].

Pancreatitis has been shown to increase intestinal permeability itself, potentially through alteration of intestinal tight junctions through decreased claudin 4 expression[35,52–54]. Theoretically, this process could enable additional systemic and pancreatic bacterial translocation, leading to a sustained state of inflammation. One study evaluated the faecal microbiota of 45 patients diagnosed with acute pancreatitis and compared the microbiota composition with 44 healthy volunteers[55]. Investigators demonstrated a greater α-diversity, a measure of the number and distribution

of different species in a given sample, in the faecal microbiota of healthy volunteers based on 16S rRNA gene sequencing compared with patients diagnosed with acute pancreatitis (TABLE 1). In addition, patients with acute pancreatitis had a greater abundance of phylum Bacteroidetes and Proteobacteria with fewer Firmicutes and Actinobacteria than those from healthy controls[55]. Whether a predominance of Bacteroidetes and Proteobacteria contributed to the necrotizing course of the pancreatitis is uncertain. Of interest is the ability to predict patients with acute pancreatitis who will subsequently develop chronic pancreatitis and its associated complications on the basis of their faecal microbiota. Given that the transition to chronic pancreatitis occurs in 20–30% of patients with alcohol as the aetiology for their pancreatitis[39,43,56], and that pancreatic necrosis is an independent risk factor for the development of chronic pancreatitis[43], investigation into potential microbial involvement in pancreatitis could prove beneficial in identifying high-risk patients.

Jandhyala and colleagues[57] evaluated the long-term functional implications of an altered intestinal microbiota in patients with chronic pancreatitis, which might give insight into downstream permutations of the intestinal microbiota related to chronic pancreatitis and their relationship to morbidity associated with this disease. Principle component analysis revealed distinct clustering of the faecal bacterial microbiota from healthy individuals as controls and those with chronic pancreatitis[57]. The investigators divided patients with chronic pancreatitis into those with and without diabetes mellitus. A significant increase in the ratio of Firmicutes to Bacteroidetes from healthy controls to patients with chronic pancreatitis but without diabetes mellitus and chronic pancreatitis with diabetes mellitus was observed between the three groups (corrected $P = 0.04$)[57]. At the species level, the relative abundance of *Faecalibacterium prausnitzii* and *Ruminococcus bromii* were both reduced from healthy controls to those with chronic pancreatitis with or without diabetes mellitus. Based on Kyoto Encyclopedia of Genes and Genomes orthology analysis, the authors observed an increase in lipopolysaccharide synthetic pathways in patients with chronic pancreatitis both with and without diabetes compared with controls. Clinically, this effect translated into an inverse relationship of *F. prausnitzii* abundance with fasting and postprandial blood glucose levels, as well as plasma endotoxin levels.

Data are limited on a microbial role in autoimmune pancreatitis (AIP) given the small proportion of chronic pancreatitis cases originating from AIP (<1 per 100,000 cases of AIP in the general Japanese population)[58–60]. In a study by Hamada and colleagues, they demonstrated a distinct difference in gut microbiota between patients with chronic pancreatitis and AIP, which might yield insight into causative factors for each condition[61]. In this study involving a limited cohort of patients (8 patients with chronic pancreatitis and 12 patients with AIP), 16S rRNA gene sequencing demonstrated a significantly increased relative abundance of *Bacteroides ovatus*, *Streptococcus australis*, *Streptococcus gordonii*, *Clostridium lactatifermentans* and *Clostridium lavalense* in chronic pancreatitis compared with AIP ($P < 0.05$). The functional consequence of this observation is currently unknown and might be a result of differences in exocrine insufficiency, which was not controlled for in their study and has been shown to alter the intestinal microbiota[62,63].

Microbiota in the development of T1DM. Diabetes mellitus is the dysregulation of glucose homeostasis, which can be a result of either insufficient insulin production by the pancreatic β-cells (T1DM) or insufficient utilization of available insulin (type 2 diabetes mellitus). T1DM is primarily an immune-mediated phenomenon in which destruction of the β-cells in the islets of Langerhans renders the patient permanently reliant on exogenous insulin to ensure glucose homeostasis[64]. The annual incidence of T1DM has increased by 3.9% in some areas of Europe, with a projected doubling in children <5 years of age between 2005 and 2020 (REFS[1,4,65]). This rising incidence highlights the role of nongenetic factors in autoimmune diseases[66,67].

Owing to known interactions between the host microbiome and immune system[68,69], investigators have studied bacterial composition associated with T1DM (TABLE 1). In an attempt to identify temporal microbial changes associated with T1DM development, the faecal microbiota of children aged 1–5 years, before the onset of T1DM, as identified by the presence of at least two diabetes mellitus-associated autoantibodies, was examined and compared with autoantibody-negative children[70]. Principle component analysis of cohorts ($n = 18$ per cohort) matched for age, human leukocyte antigen type, sex and type of formula feed (cow's milk, whey-based and casein-based formula) demonstrated that individuals with a greater number of autoantibodies to β-cells had a lower abundance of lactate-producing and butyrate-producing bacteria than those who were autoantibody negative[71]. In 2018, a longitudinal study examined microbiome changes in infants born to families with a genetic predisposition to T1DM or with first-degree relatives with T1DM. Faecal samples were collected monthly from 3 months of age until the diagnosis of T1DM was confirmed (101 T1DM cases, 267 islet autoimmunity cases and 415 controls)[72]. The faecal taxonomic composition of these children, as characterized by metagenomic sequencing, was not different between cases and controls, even with a geographically diverse cohort of patients from the USA and Europe. However, the microbiomes of healthy control children were noted to have a greater abundance of bacteria involved in the synthesis of SCFAs. These studies suggest a potential protective role for SCFAs in T1DM development given the decreased abundance of SCFA-producing bacteria associated with the diagnosis of T1DM[71,72] (FIG. 2). Although gastrointestinal dysbiosis associated with newly diagnosed cases of T1DM has demonstrated increased abundance of the genus *Bacteroides*[73,74], other studies reported a reduced abundance of butyrate-producing bacteria, again suggesting a potential beneficial effect of SCFAs in preventing T1DM development[70,75]. Utilization of nonobese diabetic (NOD) mice, which develop diabetes mellitus in a similar autoimmune fashion to humans, might provide an avenue to investigate microbial changes that alter diabetes mellitus development and pathways responsible for this phenotype. Specifically, germ-free NOD mice have been shown to have an increased serum level of IFNγ and IL-12, with concomitant lymphocyte infiltration into the islets of Langerhans, which is similar to the prediabetes mellitus state in children[76].

The interaction of gut bacteria with NOD-associated diabetes mellitus was evaluated in relation to pattern recognition receptor (PRR) signalling in NOD mice. The adapter protein MYD88 is necessary for activation of numerous PRRs, the most well-known being the Toll-like receptors (TLRs) that mediate innate immune activation in response to microbial signals[77–79]. Researchers observed that SPF-NOD-*Myd88*$^{-/-}$ mice failed to develop T1DM compared with SPF-NOD-*Myd88*$^{+/-}$ mice[80]. By contrast, germ-free NOD-*Myd88*$^{-/-}$ mice developed diabetes mellitus, which was attenuated when the mice were colonized with a defined microbial population (altered

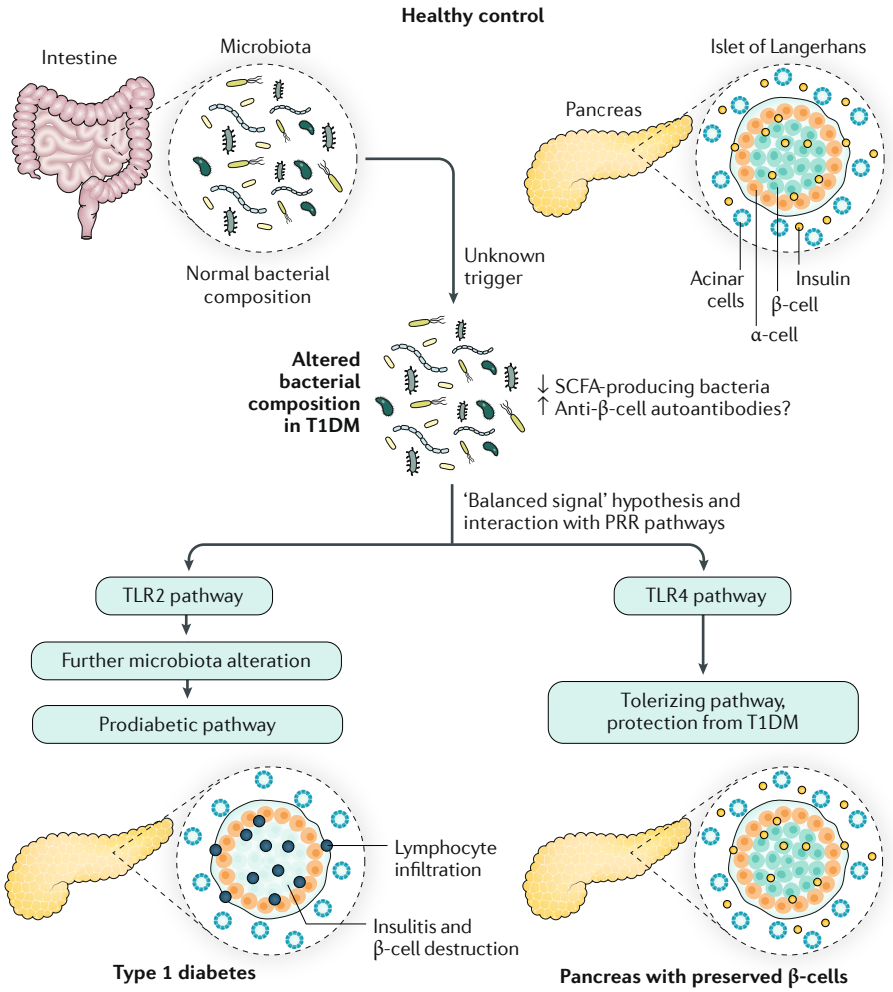

Fig. 2 | **Interactions between intestinal microbiota and the development of type 1 diabetes mellitus.** Alongside a healthy intestine with normal microbiota, a nondiseased pancreas and islet of Langerhans is illustrated with functional pancreatic β-cells producing insulin. With destruction of β-cells, patients cannot produce enough insulin for glucose homeostasis with resultant type 1 diabetes mellitus (T1DM). With a yet to be determined trigger, patients with T1DM have displayed an intestinal microbiota with lower relative abundance of short-chain fatty acid (SCFA) producers compared with healthy controls. Patients with this decrease in SCFAs, specifically butyrate-producing bacteria, demonstrate greater levels of autoantibodies to the pancreatic β-cells, which might result in β-cell destruction and development of T1DM. Microbiota also interact with pattern recognition receptors (PRRs), such as the Toll-like receptors (TLRs) 2 and 4, to modulate T1DM development through the 'balanced signal' hypothesis, in which disease development is predicated on the balance of tolerizing versus pro-inflammatory signals[84]. The TLR4 pathway, through the adapter proteins MYD88 and TRIF, has been implicated as a tolerizing pathway to attenuate T1DM development. Conversely, signalling through the TLR2 pathway alters the intestinal microbiota to facilitate a pro-T1DM phenotype. These mechanisms are thought to facilitate lymphocyte infiltration and insulitis with concomitant β-cell destruction[80,84].

Schaedler flora[81]). These findings suggest that the modulatory effect of MYD88 signalling operates in both a microbial-dependent and microbial-independent fashion. Furthermore, deletion of non-TLR bacteria-sensing pathways RIPK2 and caspase 1 or caspase 11 failed to protect mice from diabetes mellitus development, suggesting a primary role for TLR signalling in the disease[82]. Given that MYD88 is an adapter protein that is utilized for a variety of TLR receptors (except TLR3), its deletion does not implicate a specific TLR pathway[83]. Interestingly, *Tlr2* deletion favoured T1DM development in mice, suggesting that this innate sensor might capture protective signals from the microbiota as opposed to the TLR4 pathway, potentiating microbiota-mediated islet destruction[84]. Indeed, deletion of both TIR-domain-containing adapter-inducing interferon-β (*Trif*) and *Myd88* genes in mice, which are essential components of TLR4 signalling, decreased diabetes mellitus incidence and insulitis[84].

Finally, Wen et al.[80] demonstrated that *Myd88* deficiency resulted in a change in the microbiota of the distal murine gastrointestinal tract. Specifically, the ratio of Firmicutes to Bacteroidetes was lower in the NOD-*Myd88*$^{-/-}$ cohort than in NOD-*Myd88*$^{+/-}$ mice, which had an increased abundance of the bacterial families Lactobacillaceae, Porphyromonadaceae and Rikenellaceae. The authors concluded that the interaction of specific gastrointestinal bacteria with the innate immune system is a predisposing factor for T1DM development[80]. These data support a balance in the gut microbiota that regulates immune tolerance and T1DM development (FIG. 2). Dissecting microbial components responsible for this balance and interaction with specific TLR pathways might have therapeutic efficacy on T1DM prevention and treatment.

Microbiota and pancreatic cancer development: association studies. PDAC is the most common malignancy of the pancreas and is now the third most common cause of death from cancer in the USA[3,85] and predicted to be the second most common cause by 2030 (REF.[86]). As the vast majority of people are at an advanced, noncurable stage when diagnosed with PDAC, much interest exists in identifying risk factors for its development. Given that the majority of cases of PDAC are diagnosed in patients without any known risk factor other than age, such as smoking, pancreatitis or a hereditary disorder, other environmental factors might have a role[3,87]. Associative studies of oral[29,30,88], faecal[89] and organ-specific microbiota[9,10] composition have been reported in relation to PDAC (TABLE 1). Specifically, characterization of the oral microbiome focused on patients with periodontal disease as these patients had been shown to have an increased incidence of PDAC[90,91]. Farrell and colleagues, utilizing a bacterial microarray, compared the salivary microbial profile of patients with PDAC with healthy matched controls[30]. Identified bacterial candidates were then confirmed by qPCR and tested for in an independent cohort of patients with PDAC, patients with chronic pancreatitis and healthy controls. This study demonstrated a differential increase of 31 species or clusters and decrease of 25 species or clusters in patients with PDAC compared with healthy controls[30]. These changes were predominated by the phyla Firmicutes, Proteobacteria, Actinobacteria and the CFB group (*Cytophaga*, *Fusobacterium* and *Bacteroides*). Ultimately, six species were

identified as differentially represented in the saliva between healthy controls and those with PDAC, and these species were then quantified in a separate validation panel of saliva specimens from 28 patients with PDAC, 27 patients with chronic pancreatitis and 28 healthy controls[30]. Two species, *Neisseria elongata* and *Streptococcus mitis*, were significantly decreased ($P<0.05$) in PDAC compared with healthy controls, as shown by qPCR, and a logistic regression analysis suggested a potential application of these two species as PDAC biomarkers[30].

Michaud and colleagues[88] measured serum antibodies against a panel of pathogenic periodontal bacteria and correlated their presence to cases of PDAC versus healthy controls[88]. Although *N. elongata* was not one of the bacterial species tested, a correlation was noted with *P. gingivalis* (strain ATTC 53978) and PDAC. Specifically, patients with a high level of serum antibodies (>200 ng/ml versus <200 ng/ml) against this bacterium had a twofold greater association with PDAC than healthy controls[88]. A similar trend was noted in a separate study investigating survival from orodigestive cancers[92]. Finally, investigators have also characterized the presence of bacteria within pancreatic cystic neoplasms[93]. Intraductal papillary mucinous neoplasms (IPMN) are just one type of pancreatic cystic neoplasm that have variable rates of malignant transformation based on imaging risk factors and have elicited clinical interest for their potential to predict the presence of PDAC[94–97]. Gaiser and colleagues demonstrated that the bacterial copy number, as determined by 16S qPCR from bacterial DNA isolated from cyst fluid at the time of pancreatectomy, was significantly higher in IPMN associated with high-grade dysplasia and IPMN with PDAC than non-IPMN pancreatic cystic neoplasms ($P<0.005$)[93]. Although there was no difference in bacterial species richness or diversity between specimens, the pancreatic cystic microbiome did harbour oral bacterial species[93,98]. Such studies might offer insight into the development of risk factors or biomarkers for pancreatic cancer, but large population and case–control studies will be needed to validate this link.

Due to its relative ease of collection and high bacterial load, the faecal microbiota has been analysed for a variety of disease associations[99–104]. Given the anatomical continuity of the pancreas with the gastrointestinal tract, a biological deviation within the pancreas might be reflected in the microbiota downstream of the pancreas in the small or large intestine. As such, several studies have sought to characterize the faecal microbiota of patients with PDAC compared with control patients without cancer. One study by Ren and colleagues[89] prospectively evaluated the faecal microbiota of patients with PDAC from China before undergoing surgical resection ($n=85$) in comparison with healthy controls ($n=57$). The different anatomical locations of PDAC within the pancreas (head versus body or tail) might be explained by the anatomical proximity of the pancreatic head to the duodenum and the potential reflux of microorganisms into the pancreatic duct in this location, which is supported by the higher prevalence of PDAC within the pancreatic head versus body or tail[105–107]. Additionally, the clinical presentation of patients with PDAC in the pancreatic head might differ on the basis of CBD obstruction by the tumour. As such, the investigators subsequently evaluated patients based on the anatomical location of PDAC (head, $n=54$, versus body or tail, $n=31$) as well as the presence or absence of CBD obstruction ($n=22$ versus $n=32$, respectively)[89]. Although a significant difference in bacterial α-diversity was seen between healthy controls and patients with PDAC ($P<0.001$), no difference in α-diversity between PDAC in the head versus PDAC in the body or tail of the pancreas nor CBD-obstructed versus CBD-unobstructed PDAC was observed. Furthermore, the faecal microbiota of healthy controls was statistically different to that of patients with PDAC, as shown by principle coordinate analysis ($P<0.001$), but no separation between PDAC pancreas head and PDAC pancreas body or tail samples was seen, suggesting that perturbations of the faecal microbiota probably do not discriminate neoplastic transformation anatomically within the pancreas. At the phylum level, a significant increase in Bacteroidetes abundance was seen in faecal samples from patients with PDAC compared with healthy controls ($P<0.001$), and increased Firmicutes as well as Proteobacteria abundance was seen in the faecal microbiota of healthy controls versus patients with PDAC ($P<0.05$)[89]. The relative abundance of 40 genera changed between PDAC cases and controls (15 increased and 25 decreased in PDAC compared with control), and subsequent receiver operating characteristics analysis suggested predictive disease potential when using these bacterial taxa[89]. These data suggest the possibility of noninvasive diagnosis of PDAC, which currently requires invasive endoscopic techniques to secure a tissue biopsy. These findings, although intriguing, might not be applicable to patient populations outside of China, and they are potentially biased by incomplete reporting of staging data and have yet to be reproduced in broader and larger patient populations.

Several groups have recently interrogated the microbiota of the pancreas, albeit with different techniques[9,10,13]. Geller and colleagues detected bacteria within the nondiseased pancreata of organ donors and PDAC specimens by both 16S rDNA qPCR (control and PDAC) and 16S rRNA gene sequencing (PDAC samples)[13]. Bacteria were detected by qPCR in only 15% (3 of 20) of organ donor control pancreata compared with 76% (86 of 113) of PDAC samples ($P<0.005$). Taxonomic 16S rRNA gene sequencing performed on 65 of the PDAC samples demonstrated that the most abundant bacteria (51.7% of reads) belonged to the class Gammaproteobacteria and were members of the Enterobacteriaceae and Pseudomonadaceae families[13]. It should be noted that both of these families of bacteria are also members of the Proteobacteria phylum, which are present in the duodenum of healthy humans[108,109].

A proportion of patients with PDAC will present with biliary obstruction, which requires biliary stenting or decompression to relieve this clinical issue. Patients who underwent this procedure were noted to have higher amounts of bacteria within the pancreatic tumour compared with patients who did not require endoscopic decompression of their CBD and pancreatic duct[89]. The biliary stasis and bacterial overgrowth that is created by the obstruction might explain the increased amount of intratumoural bacteria in these patients given the increased abundance of intrapancreatic bacteria in patients with obstructions compared with those without obstructions[89]. Similarly, in a study by Pushalkar et al.[10], bacterial DNA content was compared between human pancreata from healthy controls and those with PDAC. This study demonstrated a significant increase in bacterial content by 16S qPCR in the PDAC samples compared with healthy controls ($P<0.0001$), although the source of the healthy control samples was not outlined. The authors further evaluated the intratumoural microbiota of PDAC specimens and compared stage I/II tumours with stage IV tumours. Although not explicitly stated, this staging likely refers to the T stage, which is based on the size of the tumour and confinement to the pancreas, given that stage IV PDAC represents metastatic disease in which patients are typically not offered surgical

resection. Compared with stage IV PDAC, stage I/II PDAC specimens were noted to have an increased abundance of Firmicutes (*Streptococcus* and *Veillonella*), whereas stage IV had increased Firmicutes (*Phascolarctobacterium*), Bacteroidetes (Paraprevotellaceae), Proteobacteria (Alcaligenaceae), and Synergistetes (Synergistaceae), although data were not corrected for false discovery rate[10].

Thomas and colleagues evaluated the pancreatic microbiota of normal (nonmalignant surgical margins), pancreatitis and PDAC human pancreatic specimens[9]. Although the authors detected bacteria in normal, pancreatitis and PDAC specimens, the microbiota did not discriminate between pathological states of the pancreas after false discovery rate correction. The bacterial genera *Acinetobacter*, *Enterobacter*, *Pseudomonas*, *Delftia*, *Enterococcus*, *Streptococcus*, *Corynebacterium*, *Propionibacterium*, *Klebsiella*, *Sphingomonas* and *Staphylococcus* were all identified in pancreatic specimens in the studies by both Thomas et al.[9] and Geller et al.[13], with *Klebsiella* being over-represented in PDAC versus normal specimens. Additionally, the phyla Proteobacteria and Firmicutes in the study by Thomas et al.[9] had a similar abundance in PDAC samples (45% and 33%, respectively) compared with the study by Pushalkar et al. (45% and 22%, respectively)[10], providing a potential future avenue of research to investigate members of these phyla in PDAC progression. Lastly, another study evaluated the intrapancreatic microbiota of human PDAC specimens and identified a microbial 'signature' that predicted short-term survivors versus long-term survivors after pancreatectomy[110]. Specifically, a significantly higher α-diversity ($P < 0.05$) and greater abundance of the genus *Saccharopolyspora* (HR 13.47, 95% CI 4.67–38.83), *Pseudoxanthomonas* (HR 5.89, CI 2.37–14.61) and *Streptomyces* (HR 4.57, CI 2.03–10.28) were found in the PDAC specimens of long-term survivors than in those of short-term survivors[110]. These studies demonstrate not only the existence of a microbiota within noncancerous pancreas but also within a neoplastic pancreas, which might differentiate disease states and be predictive of survival. Further controlled studies are needed to clarify these data.

The effect of intrapancreatic bacteria on carcinogenesis is currently an area of debate as the presence of intrapancreatic bacteria has, at present, only an associative relationship with PDAC. How these microorganisms gain access into the parenchyma of the pancreas, not simply the pancreatic ductal system, might require compromise of ductal barrier function given natural secretion of antimicrobial peptides by the pancreas and the presence of tight junction formation, as discussed earlier[12,111]. Evidence demonstrates ~68% similarity between genera of duodenal versus pancreatic duct microbiota but this similarity decreases to ~58% shared genera between pancreatic duct and pancreatic head tissue from cadaveric nondiseased pancreas[31]. The similarity decreased even further for pancreatic specimens resected for pathology (without distinction of PDAC)[31]. Although this finding provides further evidence of a pancreatic microbiota being present, if a bacterial translocation phenomenon was responsible for pancreatic diseases one would expect the bacterial similarity to increase from normal to diseased states. Regardless, the role of bacteria in PDAC development has been investigated using various preclinical models.

Microbiota and pancreatic cancer development: preclinical models. Utilizing a heterotopic mouse xenograft model of PDAC, Thomas et al.[9] demonstrated that a wide-spectrum antibiotic cocktail was able to abrogate the growth of various human PDAC cell lines, suggesting the presence of a mechanism remote of intestinal bacteria on tumour development (not intratumoural or intrapancreatic)[9]. Utilizing RNA sequencing data from harvested xenografts, the presence of a microbiota resulted in the upregulation of several procarcinogenic genes such as Tenacin-C (*TNC*), *CXCL10* and Plexin A4 (*PLXNA4*), as well as the upregulation of several genes involved in tumour suppression and patient survival such as death-associated protein kinase 2 (*DAPK2*), Krüppel-like factor 9 (*KLF9*) and Lumican (*LUM*)[9]. These findings provide evidence that the gut microbiota can induce transcriptomic changes that potentially facilitate or dampen pancreatic carcinogenesis in a distant fashion. Furthermore, gut bacteria might alter the immunological profile within PDAC that mediates pancreatic carcinogenesis. Although Thomas et al.[9] utilized NOD–SCID mice devoid of an adaptive immune system and demonstrated increased $CD45^+$ innate immune cell infiltration into PDAC xenografts from microbiota-depleted mice[9], other groups provide evidence of an adaptive immunological role in pancreatic carcinogenesis[10,21]. For example, using a heterotopic mouse model of PDAC, Sethi and colleagues showed that microbiota depletion with antibiotics resulted in an increase of antitumour immune lymphocytic cells ($CD3^+CD4^+IFN\gamma^+$, $CD3^+CD8^+IFN\gamma^+$ and $CD3^+IFN\gamma^+$) as well as a decrease in protumorigenic $CD3^+IL\text{-}17^+$ and $CD3^+CD4^+IL\text{-}10^+$ cells[21]. The antitumour effect was lost when the investigators performed heterotopic implantation in *Rag1*$^{-/-}$ mice, suggesting that the gut microbiota interact with adaptive immune cells and not innate immune cells. Importantly, in addition to antibiotics, these investigators also administered an antifungal agent twice daily in these mice. Fungi are an important component of the intestinal microbiota that affect gastrointestinal immune responses, and in 2019 they were associated with PDAC (discussed further below)[112,113]. Whether intervention to deplete fungi altered the host ability to mount an antitumour response is unclear, but it is difficult to ascertain the phenotype described by Sethi and colleagues solely on bacteria when fungi were also depleted.

Pushalkar and colleagues independently identified evidence of an altered intratumoural adaptive immune suppression utilizing the KC (*Kras*$^{LSL\text{-}G12D/+}$;*Pdx1-Cre)* genetic mouse model of PDAC and PDAC xenografts[10,114]. The germ-free KC mice demonstrated increased $CD3^+$ cell infiltration into the pancreas, which was reduced when mice were orally gavaged with faeces from either wild-type mice or tumour-bearing mice from a different genetic mouse model of PDAC, the KPC mouse (*Kras*$^{LSL\text{-}G12D/+}$;*Trp53*$^{R172H/+}$;*Pdx1-Cre*)[115]. Furthermore, isolated splenic macrophages stimulated with cell-free extract derived from faeces of KC mice decreased $CD4^+$ and $CD8^+$ lymphocyte activation compared with those derived from control faeces, as evidenced by decreased CD44 and programmed cell death protein 1 expression, as well as decreased T helper 1 cell differentiation, which is known to have an antitumour role. Finally, TLR2 and TLR5 were upregulated in the intratumoural macrophages, and activation of these receptors resulted in increased tumour growth[10]. Macrophages treated with cell-free extract from KC mice faeces, which otherwise would result in immune suppression, failed to maintain this tumour-suppressive phenotype when MYD88 signalling was ablated with an inhibitory peptide, as shown by $CD4^+$ T cell activation from increased levels of lymphocyte function-associated antigen 1 (LFA1), CD44, TNF and IFNγ compared with controls using flow cytometry. These data support a role for bacteria-induced PDAC tumour immunosuppression through TLR2 and

TLR5 signalling in tumour-associated macrophages. Potential models of bacteria interaction in PDAC development are presented in FIG. 3.

The presence of intrapancreatic bacteria raises the question of whether these microorganisms might be responsible for the ongoing issue of poor clinical response to current chemotherapeutic regimens for PDAC[116,117]. The current mainstay of treatment is gemcitabine, a pyrimidine nucleoside analogue that, when incorporated into the DNA replication machinery, results in premature termination of DNA replication and cell death[118]. Geller and colleagues, building on research that demonstrated *Mycoplasm hyorhinis* induced gemcitabine resistance, determined that isoforms of the bacterial enzyme required to metabolize gemcitabine, cytidine deaminase (CDD), were responsible for this phenotype[13]. Specifically, CDD is present in a short form (CDD_S) or a long form (CDD_L), the latter being the isoform responsible for gemcitabine metabolism. Their observation was further confirmed through xenograft experiments in which gemcitabine-mediated antitumour efficacy in mice improved when CDD-deficient *E. coli* was present compared with wild-type *E. coli* with functional CDD_L (REF.[13]). Furthermore, bacteria isolated and cultured from 14 of 15 human PDAC samples tested rendered the colon carcinoma cell lines RKO and HCT116 resistant to gemcitabine[13]. These data provide evidence that intrapancreatic bacteria, whether initiators, facilitators or bystanders of the pancreatic carcinogenesis process, are able to metabolize a common chemotherapeutic agent used to treat this disease and warrant further investigation to help explain the high chemoresistance observed clinically in patients with PDAC. Although conceptually fascinating, this study did not capture the natural history of bacterial presence in the pancreas of mice and the concomitant resistance to gemcitabine treatment as *E. coli* was artificially administered in the context of xenograft tumours. Although studies involving the interaction of the microbiome with therapeutic agents used to treat colorectal cancer[119,120], melanoma[121–123] and lung cancer[124] have been performed and suggest an ability of the microbiota to alter toxicity or efficacy, their translation to clinical use is in its infancy and randomized clinical trials are ongoing to address this translational need[125].

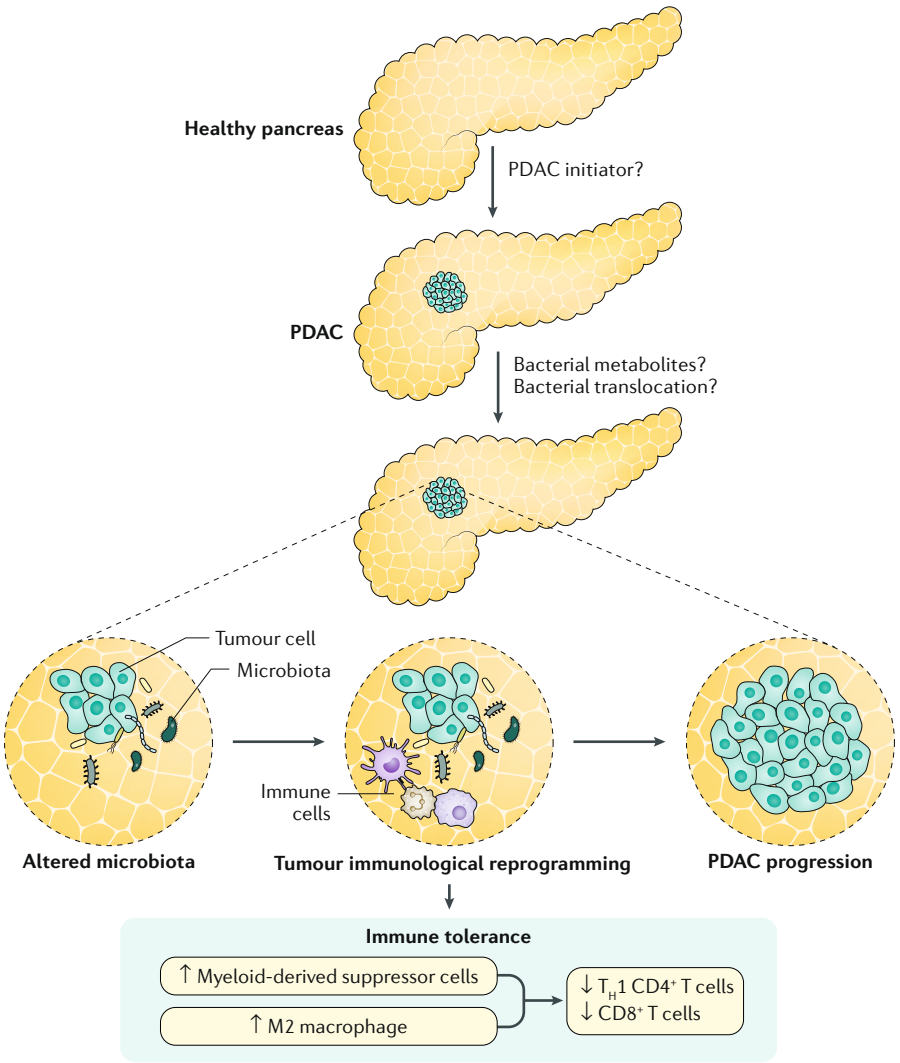

Fig. 3 | **Proposed relationship between bacteria and development of pancreatic cancer.** The oral and/or intestinal microbiota associate with the presence of pancreatic ductal adenocarcinoma (PDAC) in patients but debate exists regarding the microbial elements responsible for PDAC acceleration. Although the initiating event(s) of PDAC formation are unknown, bacteria from a distant location (that is, nonpancreatic) have been implicated in the acceleration of pancreatic carcinogenesis in mouse models[9], which might also be mediated by bacterial metabolites. Alternatively, bacterial translocation to the pancreas might occur from the intestine or oral cavity and, combined with impaired pancreatic barrier function, colonize within the pancreas to alter immune tolerance to facilitate PDAC progression. Notably, this immunological reprogramming involves increased myeloid-derived suppressor cell infiltration and transition of macrophages to an M2 subtype. These processes subsequently result in decreased $CD4^+$ T helper 1 cell (T_H1 cell) and $CD8^+$ T cell recruitment, which hinder tumour immunosurveillance with subsequent PDAC progression.

Future directions

A new front in pancreatic research has opened with the observation that alterations in oral and intestinal bacterial composition are associated with both benign and malignant pancreatic diseases (TABLE 1). Although a number of these studies showed associative correlation between bacteria and pathology, some investigators demonstrated functional implications of bacteria in pancreatic cancer using preclinical models. As the evidence mounts on the effect of bacteria in pancreatic diseases, which could be mediated through altered innate signalling and immunological responses, a number of questions remain unanswered.

First, the emerging link between intestinal microbiota and pancreatic health highlights the concept of a local versus remote (that is, nonlocal) effect of bacteria on organ physiology. By which mechanisms might the oral or intestinal bacteria alter pancreatic health via nonlocal effects? Indeed, bacterial translocation into the pancreas from the oral or intestinal compartment can initiate important immune alterations in this organ as highlighted by Pushalkar et al.[10]. However, the events leading to bacterial translocation

into the pancreas remain unclear, especially given that healthy mice are devoid of pancreatic bacteria, even in the presence of intestinal inflammation[9]. The pancreas might be a privileged organ whereby pancreatic ductal epithelial tight junctions and antimicrobial peptide secretion provide a barrier function to the translocation of bacteria and organ colonization[12,111]. A disturbance in these local defence mechanisms might render the pancreas more susceptible to opportunistic bacterial colonization and change in immune environment. This model would imply a defective barrier function at the original oral or intestinal site as well as potentially within the pancreas itself, all of which have been shown experimentally and clinically[35,52,126].

Alternatively, and not mutually exclusive to the translocation model, microbially derived small molecules can alter pancreas immune function, thereby contributing to inflammation and carcinogenesis. For example, studies using germ-free and conventionally raised mice showed that microbiota affect the production of thousands of metabolites present in the serum and various organs, including the liver, brain and heart[127]. Some of these metabolites, such as SCFAs, bile acids and indole derivatives, have important immunomodulatory functions and actively participate in host–microbiota interactions[128]. It should be stressed that the vast majority of microbially derived metabolites and potential host targets remain unidentified, and it is likely that novel molecules and targets will be identified as technology evolves. For example, forward genetic screens using specific host signalling pathways could be used against the bacterial consortia associated with disease states. Such an approach was used to screen bacteria-derived metabolites that activate G protein-coupled receptors that were produced by an inflammatory bowel disease (IBD)-associated bacterial consortium[129]. Thus, it is conceivable that intestinal bacteria-derived small molecules might alter the pancreatic tumour microenvironment through activation of specific immune and nonimmune cell receptors. Furthermore, these bacteria-derived small molecules might be more than just modulators of a pre-existing pancreatic tumour microenvironment and could be capable of initiating pancreatic carcinogenesis through the action of genotoxins as in colorectal cancer[130]. This model of remote bacterial influence is compatible with data generated from xenograft models, which are typically devoid of bacteria[9]. The physiological conditions regulating the production of these microbially derived bioactive molecules and their specific function in the pancreas is unknown and additional investigation would be needed to move this field of research forwards.

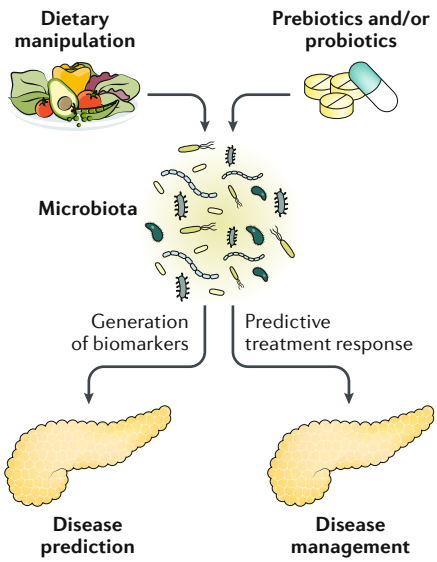

Fig. 4 | **Areas of clinical interest to modulate microbiota influence on pancreatic disease.** Given evidence of the microbiota impacting pancreatic diseases, altering the microbiota via dietary manipulation or administration of prebiotics or probiotics might shift the microbiota to mitigate risk. With increasing interest in the microbiota and pancreatic disease, biomarkers could be discovered to predict disease. Finally, the microbiota might enable clinicians to predict treatment response to drugs, such as chemotherapy and diabetic medications, to guide disease management.

The implication of microbiota in pancreatic cancer provides potential therapeutic options such as the generation of biomarkers or microbial signatures that predict response to neoadjuvant and adjuvant therapies (FIG. 4). Although metabolism of chemotherapeutic drugs might be relevant to anticancer response[13], a lack of data exist linking antibiotic usage and cancer survival of patients to therapeutics. The therapeutic success of immunotherapy in patients with advanced melanoma and lung cancer, in conjunction with emerging evidence that intestinal bacteria might define the state of drug responsiveness at least for immune checkpoint blockade, leads to the question of whether a similar role for bacteria exists in pancreatic cancer[131,132]. However, microbial signatures defining immune checkpoint responsiveness remain elusive[123] and so the feasibility of using microbiota as predictors of drug efficacy for PDAC is debatable. Addressing this question would require establishment of immune checkpoint blockade efficacy in patients with PDAC, which at present has not demonstrated efficacy in clinical trials[133,134]. Manipulation of the gut microbiota using diet-based interventions, prebiotics and/or probiotics might also be a modality that could affect pancreatic disease (FIG. 4).

However, a multicentre, randomized, double-blind placebo-controlled trial to test whether prophylactic administration of probiotics could diminish infectious complications in patients with acute pancreatitis not only showed a failure to reduce the risk of infectious complications but was associated with a 2.5-fold increased risk of mortality (relative risk 2.53, 95% CI 1.22–5.25)[135]. Thus, more investigation is necessary before microbiota manipulation can translate into clinical outcomes. Moreover, as pancreatic bacterial diversity associates with long-term survival of patients with PDAC, the mere presence of intratumour bacteria might not always be synonymous with bad outcomes[110]. Thus, a complex interaction between intrapancreatic bacteria and disease outcomes exists in which, on the one hand, some bacteria appear to promote PDAC development[13] while, on the other hand, bacterial diversity influences patient survival[110]. Untangling these complex interactions requires further investigation.

Up to this point, the virome and the mycobiome have been unexplored in PDAC development but have been linked to the development of colorectal cancer[136,137]. However, a study in 2019 has provided evidence that the mycobiome might also contribute to PDAC development as distinct mycobiomes were noted between PDAC tumours and those of the gut or normal pancreas. Ablation of the mycobiome protected against PDAC progression, similar to models related to antibiotics and the microbiome[113]. Additionally, fungus-associated PDAC progression might be secondary to ligation of mannose-binding lectin, an activator of the complement cascade following binding to glycans on fungal cell walls[113]. This line of investigation is in its infancy but underlines the importance of dissecting the role of viruses, fungi and bacteria, as well as their interactions, in pancreatic diseases.

Conclusions

In conclusion, a new front in microbiota host response research has opened with the evidence for a bacterial role in pancreatic diseases. Although mechanistic understanding of this relationship is still limited, it is clear that this field of research is

moving forwards and that novel therapeutic interventions based on bacteria-related function could be generated in the near future.

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