4

Epidemiology in the human body

4.1 Analogy to COVID-19

The COVID-19 pandemic has facilitated public understanding of epidemiology and contact-tracing. Besides exchanging microbes with the environment on a daily basis, the human microbiome also involves movements of microbes within the body and sometimes into human cells. From an ecological point-of-view (Chapter 2), the microbiome at a given body site is a result of multiple source communities, and individual-specific factors help shape the steady-state abundance of microbes. Controlling the source and improving the local condition would lead to more effective treatment.

We discussed sample collection in Chapter 3, and the same principles would apply to all kinds of samples that may need to be collected to track down the disease-relevant microbes. Successful examples of comprehensive studies are as yet scarce, but we will hopefully have more to talk about in the years to come.

Worked sample 4.1

If you are traveling to a remote village, what samples from the environment and from the locals do you plan to study, before you look for these new microbes in your own microbiome? After how many days shall we expect to see a change in the skin, oral, or the fecal microbiome?

Worked sample 4.2

As we have mentioned in Chapter 3 and learned through experience with the COVID-19 pandemic, humans constantly contaminate the environment with the microbes we carry. This Worked Sample is also a warm-up for more on taxonomy in Chapter 5.

Investigating Human Diseases with the Microbiome: Metagenomics Bench to Bedside.
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The following study on the microbiome in New York subways (Fig. 4.1) had to rely on HMP data back then [1]. Can we better assign the metagenomic data to the most likely human body sites now? Shall we consider data from different ethnic groups in the analyses of some stations? What is now the closest match for the *Pseudoalteromonas* species identified in the previously flooded station?

4.2 Sources of potential pathogens in the infant gut

When a baby gets diarrhea, and we are lucky enough to catch some samples for metagenomic shotgun sequencing, is the diarrhea due to something that the baby ate or drank [2]? Was there a change in formula milk (contains bacterial spores from cows, in addition to nutrients)? Will direct breastfeeding be better than pumping milk [3]? Shall we pay more attention to the mother's gut microbiome (Fig. 4.2)? In addition to the transmission through the environment, some of the mother's gut microbes could somehow get to the mammary gland and become part of the breast milk (~ 10^6 cells/mL bacteria, ~ 3×10^5 cells/mL fungi according to PCR (polymerase chain reaction) [4,5]). Babies often spit up milk along with microbes back to the mother, so the baby's own oral microbiome is also part of the loop (Fig. 4.2). Oral-gut transmission is also more common in infants than in adults [6]. When only breast milk and areolar skin (nipple ring) microbiota from the mother are analyzed by amplicon sequencing, bacteria from breast milk contributed nearly 30% of the infant gut microbiota in the first month for infants who were more than 75% breast-fed, dropping below 10% contribution after the first month of age [7].

4.3 Ectopic presence of commensal microbes

Many of the microbes in the human body may be harmless if they stay where they belong. However, due to a rare occasion (to be captured for a more complete understanding, Chapter 8), or due to a routine process that is defended against under normal conditions, the microbes may stay and grow in the wrong place.

Colonization of salivary bacteria from Crohn's disease or Ulcerative Colitis patients (two major types of Inflammatory Bowel Diseases (IBDs)) into the gut of germfree mice has been shown to induce inflammation [8]. Some of the human disease biomarkers identified by MWAS are likely of oral origin, and their colonization in the gut could involve genetic factors [9,10], in addition to age and other physiological conditions.

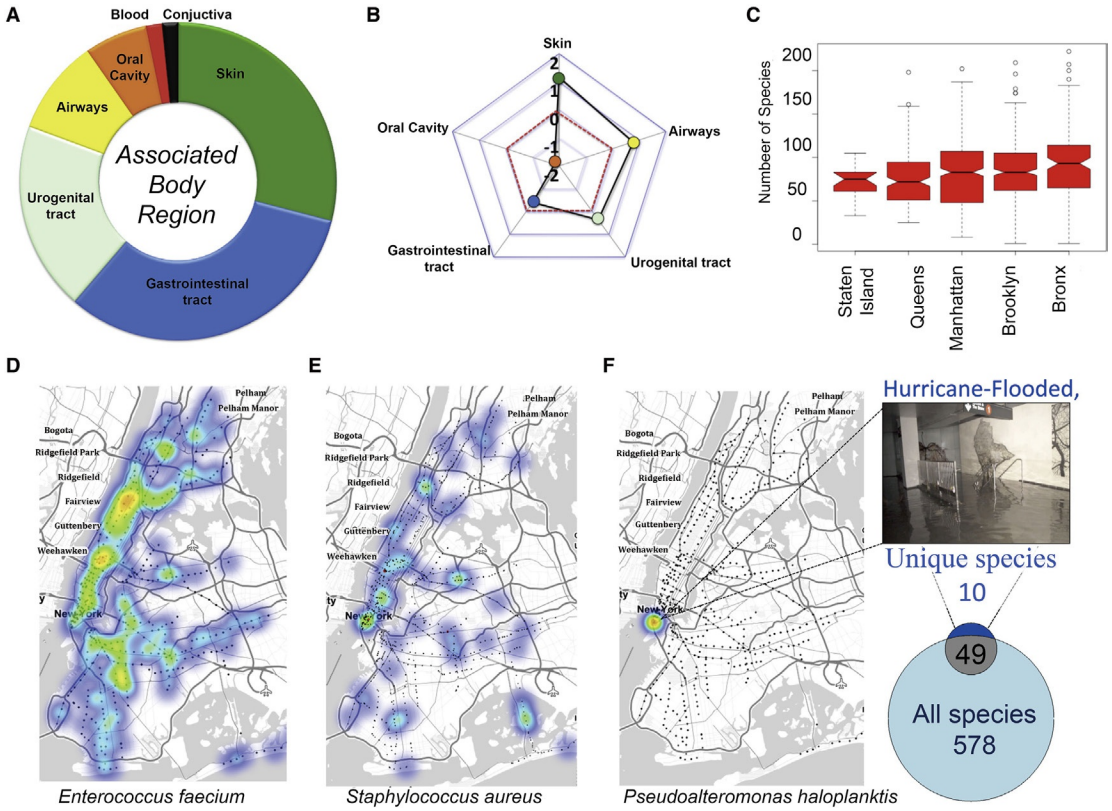

Fig. 4.1 Taxa diversity and the likely human body sites for the NYC subway swabs. Detected bacteria were annotated relative to the most commonly associated body part from the Human Microbiome Project (HMP) dataset. (A) Of the 67 PathoMap species that matched the HMP dataset, the proportions were greatest for the GI tract *(blue)*, skin *(green)*, and urogenital tract *(white)*. The entire circle represents 100% of the 67 species, and the sizes of each color represent the proportion of each type of bacteria. (B) To account for the database proportions from the HMP, we calculated the log2 of the observed versus expected numbers of species found for each category, which indicated that skin was the most predominant type of bacteria on the subway system. (C) Boxplot of the number of species found per borough. Middle line of each section shows the median, and the top and bottom of each box show the 75th and 25th percentiles, respectively. Notches show a significant difference between groups (95% confidence interval). (D and E) Heatmaps of NYC showing the density for *Enterococcus faecium* (D) and *Staphylococcus aureus* (E). Small red dots indicate the presence of a fully resequenced mecA gene. (F) Analysis of a subway station (picture on top shows the station) flooded during Hurricane Sandy. The Venn diagram compares the unique set of 10 species in the data from that station that did not appear in any other station or area of NYC, but 52 species overlapped with the set of 627 species present in the subway system. (A–F) The entire NYC MTA subway system, a total of 468 stations, was swabbed in triplicate over the course of the summer of 2013 and some additional samples taken for culturing and testing and in response to reviewers in 2014. Two surfaces were swabbed in each station, and one surface was swabbed within the train. Samples were collected from turnstiles and emergency exits, Metro Card kiosks, wooden and metal benches, stairwell handrails, and trashcans. The turnstiles and kiosks were prioritized at each station due to the level of human-surface interaction at these particular sites. In the train, the doors, poles, handrails, and seats were swabbed. Credit: Fig. 5 of Afshinnekoo E., Meydan C., Chowdhury S., Jaroudi D., Boyer C., Bernstein N., et al. Geospatial resolution of human and bacterial diversity with city-scale metagenomics. Cell Syst 2015;1:1–15. doi:10.1016/j.cels.2015.01.001.

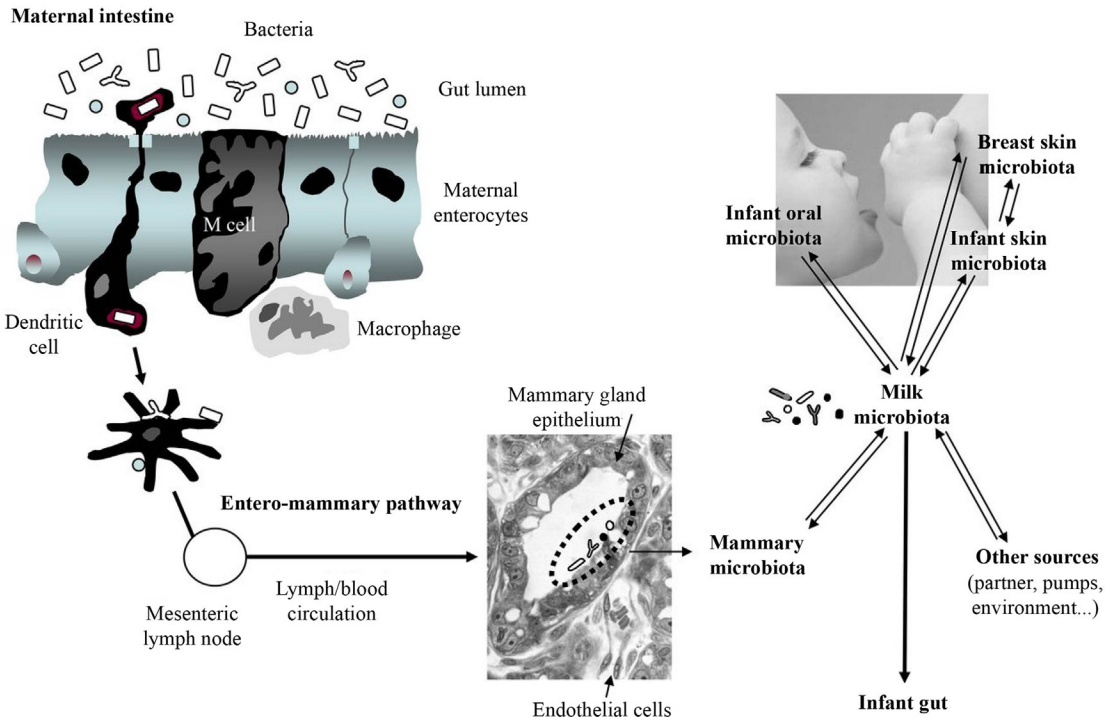

Fig. 4.2 Sources of the bacteria present in human milk, including a model to explain how some maternal bacterial strains could be transferred to the infant's gut through an entero-mammary pathway. Credit: Fig. 1 of Rodríguez JM. The origin of human milk bacteria: is there a bacterial entero-mammary pathway during late pregnancy and lactation? Adv Nutr 2014;5:779–84. https://doi.org/10.3945/an.114.007229.

Translocation of gut bacteria into secondary lymph nodes has been studied in mouse models of cancer, which could facilitate immune response and treatment (Chapter 7, Table 7.2).

Impaired gut epithelium barrier function (leaky gut) with insufficient butyrate production has been implicated in many diseases, while the inflammation is typically attributed to LPS (lipopolysaccharides). Bacteria DNA has been detected in the blood (e.g., Refs. [11–13]), but we do not know whether some of these bacteria may be alive in healthy adults (occult sepsis), e.g., hiding inside immune cells like some well-studied pathogens. For example, *Streptococcus pneumonia* is able to replicate within splenic macrophages [14]. *Streptococcus pyogenes* has been shown to remain extracellular, when transiting between multiple lymph nodes in lymphatic vessels to enter the bloodstream [15]. Vancomycin-resistant *Enterococcus* (VRE) is known to dominate the gut microbiome before bloodstream infection (sepsis) in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HCT) [16]. *Candida* spp. fungi have also been shown to expand in the gut of allo-HCT patients before they cause infection in the blood (Fig. 4.3; Remember the microbial cell number question in Chapter 1).

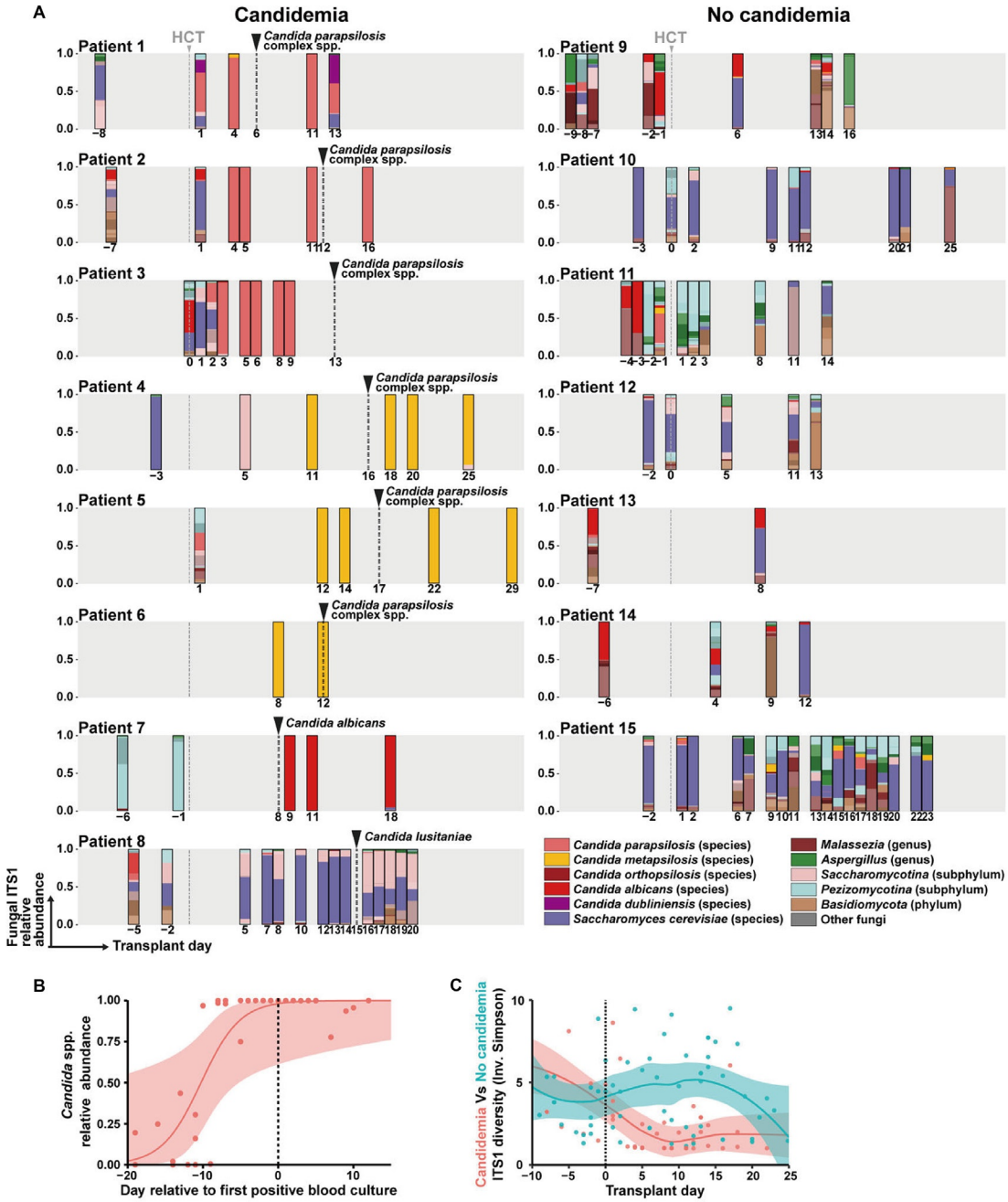

Fig. 4.3 Mycobiota dynamics in allo-HCT patients. (A) Species-level taxonomy of fecal mycobiota (average: 7 samples per patient, range: 2–18) from allo-HCT patients with (left column) and without (right column) candidemia, colored according to the legend. Frequent species, for example, *Candida* spp. and *Saccharomyces cerevisiae*, were individually color coded. The *gray box* indicates day −10 to day +30 of transplantation and the *gray dashed line* indicates the day of transplantation. The number below each bar graph indicates the day of sampling. The *black dashed line* and *arrow* indicate the day of the first fungal bloodstream infection in the candidemia group. (B) Quantification of total relative abundance of pathogenic *Candida* spp. of each fecal sample (n=37) from patient 1 to patient 7; a *solid line* represents the dynamic trend, with the *shaded area* indicating the 95% confidence interval. (C) α-Diversity of mycobiota in each sample, measured by the inverse Simpson index. *Red dots and line*: candidemia group (n=51); *turquoise dots and line*: noncandidemia group (n=57). Credit: Fig. 2 of Zhai B, Ola M, Rolling T, Tosini NL, Joshowitz S, Littmann ER, et al. High-resolution mycobiota analysis reveals dynamic intestinal translocation preceding invasive candidiasis. Nat Med 2020;26:59–64. https://doi.org/10.1038/s41591-019-0709-7.

Besides, germfree mice show impaired blood-brain barrier (BBB) function, which could be restored with butyrate, *Clostridium tyrobutyricum*, or *Bacteroides thetaiotaomicron* (produces the other major SCFAs, acetate and propionate) [17]. The hormonal cycle also impacts BBB, with estrogen being protective [18] (More on the menstrual cycle in Chapter 8). There are plenty of things to consider for the gut-brain axis.

4.4 Get to where it matters for the disease

4.4.1 Rheumatoid arthritis

The fecal, saliva, and dental microbiomes have been studied for rheumatoid arthritis (RA), and likely contribute to immune derangement [19,20]. The relative abundances of *Lactobacillus salivarius* correlated between the oral and the fecal samples (Fig. 4.2), and the RA-enriched strain likely differed from the known probiotic *Lactobacillus salivarius*; *Lactobacillus* had been reported to be enriched in patients with xerostomia (dry mouth) [19]. Oral *Prevotella* and *Veillonella* are more abundant in smokers [21].

The lung microbiome, although relevant for the effect of smoking on RA and for RA comorbidities, requires invasive bronchoalveolar lavage (BAL) sampling and is usually not investigated. A study of 20 new-onset RA patients, 10 sarcoidosis patients, and 28 healthy controls found BAL *Veillonella* to correlate with immunoglobulin A (IgA) against cyclic citrullinated peptides (CCP) and IgA against rheumatoid factor (RF) [22], consistent with oral microbiome results from a different cohort [19]. An unclassified Oxalobacteraceae negatively correlated with DAS28 (Disease Activity Score), reminiscent of a bacterium close to *Oxalobacter formigenes* that was relatively depleted in the fecal microbiome of RA patients compared to controls in a separate study [19].

Evidence for bacteria in the synovial fluid is available in the literature. PCR (polymerase chain reaction) against select dental bacteria in culture-negative synovial fluid samples frequently detected bacteria, while leukocytes were PCR negative [23]. For example, *Prevotella intermedia* was detected in 19/19 dental plaques, 14/19 serum, and 17/19 synovial fluid of patients with refractory RA (medication status unknown); *Porphyromonas gingivalis* was detected in 15/19 dental plaques, 8/19 serum, and 11/19 synovial fluid samples of refractory RA; *Aggregatibacter actinomycetemcomitans* was detected in 4/19 dental plaques, 0/19 serum, and 3/19 synovial fluid samples of refractory RA [23]. A 16S rRNA gene amplicon study on 110 synovial fluid samples from RA patients and 42 synovial fluid samples from osteoarthritis (OA) patients, identified *Veillonella dispar*, *Haemophilus parainfluenzae*, *Prevotella copri*, *Atopobium* sp. and *Treponema amylovorum* to be more abundant in RA, and *Bacteroides caccae* to be

more abundant in OA [24]. These RA synovial fluid-enriched bacteria (or another species in the same genus) have all been previously reported as significant biomarkers in the gut or oral microbiome in RA patients or controls [19,20,25,26]. All these synovial fluid samples were culture-negative according to standard microbiology practices. The study also collected synovial tissue samples, and the results did not overlap with the synovial fluid bacterial biomarkers [24].

Aggregatibacter actinomycetemcomitans, but not other better-known periodontitis pathogens (*Porphyromonas gingivalis*, *Tannerella forsynthia*, *Treponema denticola*, *Fusobacterium nucleatum*, *Parvimonas micra*, *Prevotella intermedia*), triggered hypercitrullination (to proteins) in neutrophils, through a pore-forming toxin leukotoxin A (LtxA) expressed by *A. actinomycetemcomitans* [26]. Antibody against LtxA was enriched in RA patients and in periodontitis patients and showed some overlap with anticitrullinated protein antibodies (ACPA)-positive and with rheumatoid factor (RF)-positive RA [26]. LPS (lipopolysaccharide) from the periodontal bacteria *Aggregatibacter actinomycetemcomitans* (renamed from *Actinobacillus actinomycetemcomitans*), *P. intermedia*, and *Porphyromonas gingivalis* has also tested positive for their ability to induce osteoclast differentiation in vitro [27], potentially contributing to bone erosion, a debilitating aspect of RA progression.

In addition to Th17 (T helper 17) cell activation [28], recent single-cell studies indicated the recruitment of neutrophils and other immune cells by oral mucosal fibroblasts in periodontitis [29]. Neutrophils are the major population in White Blood Cell (WBC) counts reported by routine blood tests and showed positive associations with RA-enriched microbes such as *Lactobacillus salivarius*, *Veillonella* spp. and negative associations with control-enriched microbes such as *Lactococcus* sp. [19] (Fig. 4.4).

4.4.2 Cardiometabolic diseases

Patients with liver cirrhosis have been reported to show an overabundance of potentially oral bacteria in feces, which were relieved after withdraw of proton pump inhibitors (PPI, e.g., omeprazole) [30–33]. Yet we do not know whether or not these bacteria are in the liver. PPI use would allow more salivary bacteria to survive the stomach, and increased the relative abundance of fecal *Streptococcus*, in correlation with increased serum gastrin level [33]. Periodontal therapy showed favorable results in a group of cirrhosis patients [34].

Many cardiovascular events could be linked to a dental cleaning. A myriad of bacteria has been reported in atherosclerotic plaques [35] (Table 4.1), all of which can be part of the oral microbiome [77,78]. Atherosclerotic plaque species such as *Klebsiella pneumoniae* can

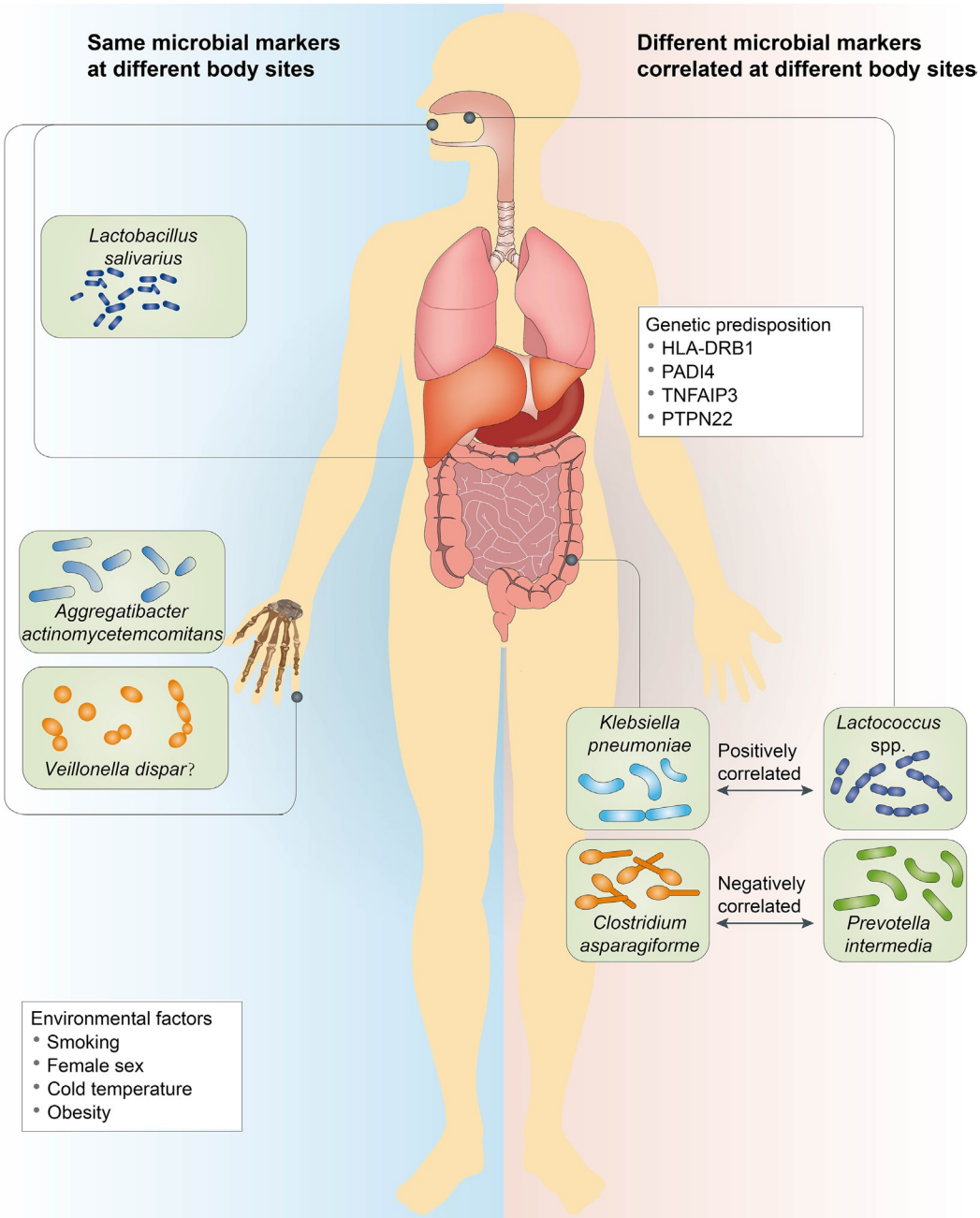

Fig. 4.4 Microbiome disturbances in rheumatoid arthritis. Oral and fecal samples are noninvasive, while samples from the joints or from the lungs are more difficult to obtain. This would also be a question for immune cell populations. The synovial fluid results are not yet shotgun metagenomics and are not matched with oral metagenomic data from the same patients. Credit: Added two bacteria onto Fig. 4 of Wang J, Jia H. Metagenome-wide association studies: fine-mining the microbiome. Nat Rev Microbiol 2016;14:508–22. https://doi.org/10.1038/nrmicro.2016.83, Huijue Jia, Chen Chen of BGI-Shenzhen.

be of either gut or oral origin [49], which contribute to LPS and TMA (trimethylamine, metabolized into TMAO (trimethylamine-*N*-oxide) in the liver implicated in atherosclerotic cardiovascular diseases [79,80]. More personalized monitoring of the microbiome would be recommended (Chapter 8).

Table 4.1 Atherosclerotic plaque-associated bacteria and methods of detection.

Atherosclerotic plaque-associated bacteria	Detection platform	Percentage of bacteria present in atherosclerotic plaque samples	Reference
Aggregatibacter actinomycetemcomitans [Phylum: Proteobacteria]	PCR	71.4% [5/7]	[36]
	16S rRNA	66.67% [28/42]	[37,38]
	mAb	17% [5/29]	[39,40]
	16S rRNA	21.87% [7/32]	[41]
	16S rRNA	18% [9/50]	[42]
	16S rRNA	25.9% [7/27]	[43]
	RT-PCR	46.2% [18/39]	[44,45]
	16S rRNA	29.4% [15/51]	[46,47]
Chlamydiae *pneumoniae* [Phylum: Chlamydiae]	mAb	20.6% [6/29]	[39,40]
	16S rRNA	35.4% [11/31]	[39,41]
	16S rDNA	18% [9/50]	[39,42]
	ICC/PCR	48% [11/23]	[48]
	16S rRNA	51.5% [17/33]	[49]
	MIF IgA	32.6% [63/193]	[50]
	MIF IgG	61.7% [119/193]	[50]
	16S rRNA	26% [12/46]	[51]
	PCR	42% [102/241 sections (10 samples)]	[52]
	PCR	69% [11/16]	[53]
	Immunofluorescence	79% [71/90]	[54]
	PCR	70% [42/60]	[55]
	IgG antibody	61.7% [50/81]	[56]
Campylobacter rectus [Phylum: Proteobacteria]	16S rRNA	9.52% [4/42]	[37,38,57]
	PCR	11.7% [6/51]	[44,46]
	16S rRNA	21.51% [11/51]	[44,46,58]
	16S rRNA	15.7% [8/51]	[59]
	16S rRNA	21.51% [11/51]	[43]
Enterobacter hormaechei [Phylum: Proteobacteria]	16S rRNA	50% [134/268]	[60]
	16S rRNA	40% [2/5]	[61]

Continued

Table 4.1 Atherosclerotic plaque-associated bacteria and methods of detection.—cont'd

Atherosclerotic plaque-associated bacteria	Detection platform	Percentage of bacteria present in atherosclerotic plaque samples	Reference
Eikenella corrodens [Phylum: Proteobacteria]	16S rRNA	54.76% [23/42]	[37,38]
	PCR	15.6% [8/51]	[57]
	16S rRNA	27.45% [14/51]	[59]
Fusobacterium nucleatum [Phylum: Fusobacteria]	16S rRNA	50% [21/42]	[37,38]
	Monoclonal antibody	34% [10/29]	[39,40]
	PCR	21% [4/19]	[62]
Fusobacterium necrophorum [Phylum: Fusobacteria]	–	–	[63–65]
Helicobacter pylori [Phylum: Proteobacteria]	IgA	55.4% [107/193]	[50]
	IgM	44.6% [86/193]	[50]
	16S rRNA	37% [17/46]	[51]
	IHC	57.8% [22/38]	[66]
	PCR	92.16% [47/51]	[67]
	IgG	67.9% [55/81]	[56]
Mycoplasma pneumoniae [Phylum: Tenericutes]	Seropositivity	14% [396]	[68]
	–	–	[69]
Porphyromonas endodontalis [Phylum: Bacteriodetes]	–	–	[70]
Porphyromonas gingivalis [Phylum: Bacteriodetes]	16S rRNA	78.57% [33/42]	[37,38]
	PCR	71.43% [5/7]	[36]
	16S rRNA	67% [134]	[60]
	mAb	52% [15/29]	[39,40]
	16S rRNA	22.27% [6/22]	[39,41]
	16S rRNA	26% [13/50]	[39,42]
	PCR	47.4% [9/19]	[62]
	PCR	51% [27/53]	[71,72]
	PCR	43.1% [22/51]	[57]
	16S rRNA	45.1% [23/51]	[44,46]
	16S rRNA	21.6% [11/51]	[44,46,58]
	RT-PCR	53.8% [21/39]	[44,45]
	16S rRNA	45.1% [23/51]	[59]
	16S rRNA	7.4% [2/27]	[43]
Prevotella intermedia [Phylum: Bacteroidetes]	mAb	41% [12/29]	[39,40]
	16S rRNA	9.37% [3/32]	[39,41]
	16S rRNA	14% [7/50]	[39,42]
	PCR	21% [4/19]	[62]
	PCR	15% [8/53]	[72,73]
	PCR	19.6% [10/51]	[57]
	RT-PCR	79.3% [23/29]	[44,45]
	PCR	71.43% [5/7]	[36]
	16S rRNA	3.7% [1/27]	[43]

Table 4.1 Atherosclerotic plaque-associated bacteria and methods of detection.—cont'd

Atherosclerotic plaque-associated bacteria	Detection platform	Percentage of bacteria present in atherosclerotic plaque samples	Reference
Prevotella nigrescens [Phylum: Bacteriodetes]	PCR	15.6% [8/51]	[57]
	RT-PCR	17.9% [7/39]	[44,45]
Pseudomonas aeruginosa [Phylum: Proteobacteria]	16S rRNA	40% [6/15]	[74][a]
Pseudomonas luteola [Phylum: Proteobacteria]	16S rRNA	100% [15/15]	[75]
Streptococcus gordonii	PCR	19.4% [–]	[43][b]
Streptococcus mitis	PCR	19.4% [–]	
Streptococcus mutans	PCR	74.1% [20/27]	
Streptococcus oralis	PCR	3.7% [1/27]	
Streptococcus sanguinis [Phylum: Firmicutes]	PCR	25.9% [7/27]	
Treponema denticola [Phylum: Spirochaetes]	PCR	43% [23/53]	[44,71]
	16S rRNA	44.4% [12/27]	[43]
	PCR	35.2% [18/51]	[57]
	16S rRNA	49.01% [25/51]	[44,46]
	16S rRNA	27.4% [14/51]	[44,46,47]
	16S rRNA	23.1% [6/26]	[43,47]
	16S rRNA	49.01% [25/51]	[59]
Tannerella forsythia [Phylum: Bacteriodetes]	16S rRNA	61.9% [26/42]	[37]
	PCR	100% [7/7]	[36]
	mAb	34% [10/29]	[39,40]
	16S rRNA	30% [15/50]	[39,42]
	PCR	10.5% [2/19]	[62]
	PCR	19.6% [10/51]	[57]
	16S rRNA	5.9% [3/51]	[38,44,46]
	RT-PCR	25.6% [10/39]	[44,45]
Veillonella [Phylum: Firmicutes]	16S rRNA	10% [2/20]	[76]
	16S rRNA	100% [13/13]	[75]

[a]The author did not find this result in the review and did not receive a response from [35].
[b]The original table from [35] indicated 16S rRNA, which was used for other bacteria in [43]; Streptococcus species were distinguished from one another using PCR against the glucosyltransferase gene [43].
Credit: From Table 1 of Chhibber-Goel J, Singhal V, Bhowmik D, Vivek R, Parakh N, Bhargava B, et al. Linkages between oral commensal bacteria and atherosclerotic plaques in coronary artery disease patients. NPJ Biofilms Microbiomes 2016;2:7. https://doi.org/10.1038/s41522-016-0009-7.

Members of the human saliva microbiome and subgingival microbiome have been found in mouse placenta following injection through the tail vein [81]. In addition to human cases of preterm birth and term stillbirth, *Fusobacterium* has also been implicated in preeclampsia. Mice transplanted with feces from preeclampsia cases showed bacteria including *Fusobacterium* in their placenta, along with elevated expression of proinflammatory cytokines and chemokines such as IL-6 (interleukin-6), IL-1b, Ccl3 (CC-type chemokine 3), Ccl4 [82].

Lactobacillus crispatus is a dominant bacterium in the healthy vagina correlated with hormones including testosterone [83,84], and its abundance in the fecal microbiome correlated with that in the vagina [85]. However, *Lactobacillus crispatus* together with other Lactobacilli appeared more abundant in the fecal microbiome of atherosclerotic cardiovascular disease (ACVD) patients (Chapter 2, Fig. 2.14); the *Lactobacillus crispatus*-dominant vaginal microbiome was more prevalent in women who used statin, compared to those with high cholesterol who did not take statin and to those with normal cholesterol [86]. Further study would be needed for the potential modulation of *Lactobacillus crispatus* by statin in cardiovascular diseases, and the likely sex difference in *Lactobacillus crispatus* distribution in the human body.

Worked sample 4.3

With your practical and theoretical knowledge of the microbiome from the previous Chapters, what question would you like to investigate for the lungs (Fig. 4.5), and how would you design the collection of samples and other information?

4.5 Interkingdom interactions in the microbiome in diseases

Commensal bacteria typically prefer a neutral pH and body temperature, while fungi can tolerate lower pH, dryness, and other not so encouraging conditions. Unfortunately, amplicon sequencing for bacteria would not detect fungi, and amplicon sequencing for fungi would not detect bacteria (Chapter 3, Fig. 3.4). Judging from shotgun metagenomic data of a typical sequencing amount (if the samples were properly extracted), fungi are usually of low abundance in the gut, vagina, and mouth of healthy individuals [85,87,88], while being better known on the skin [89]. Mycobiota dysbiosis is seen in infectious diseases and other conditions involving various body sites (Fig. 4.6). Perhaps with more data, we would be able to predict a fungal boom when it is

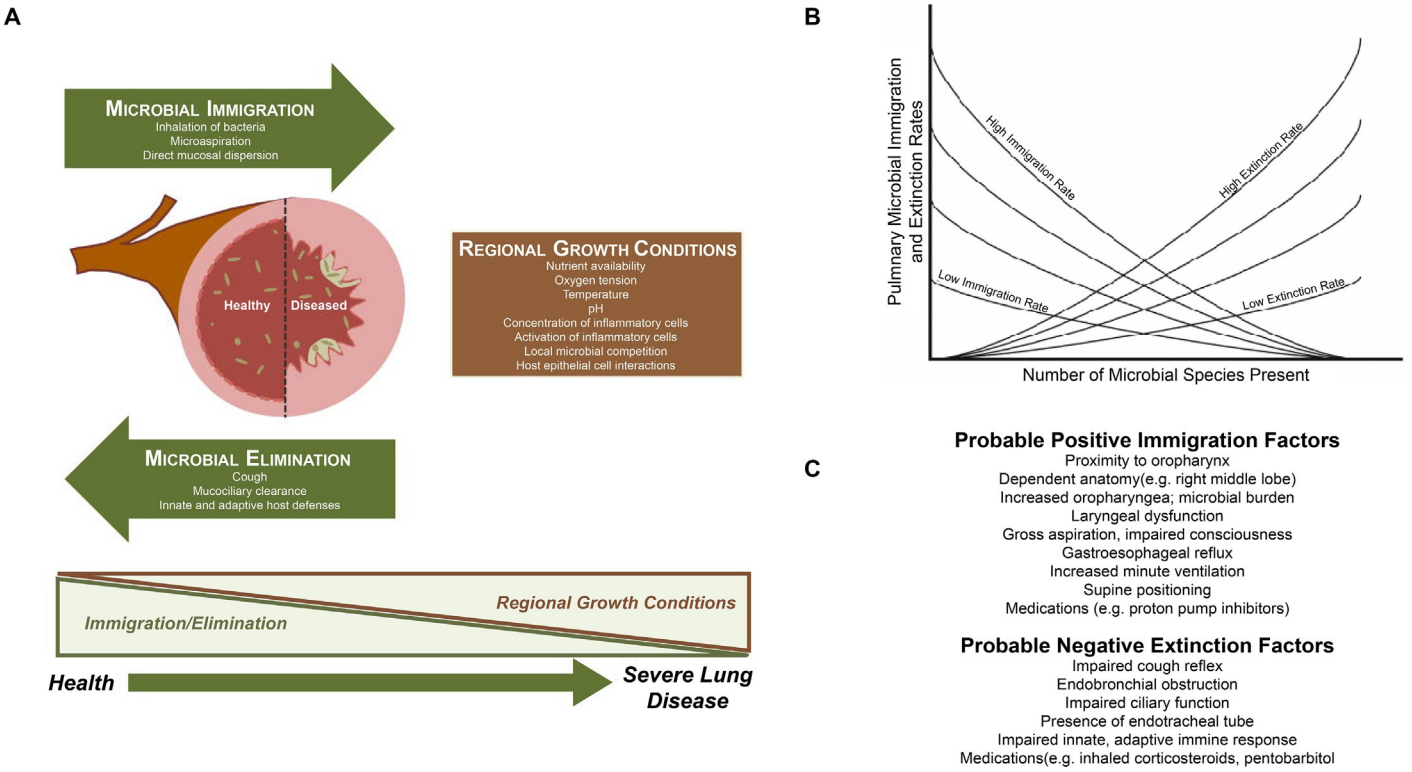

Fig. 4.5 Ecological modeling of the respiratory microbiome. (A) The constitution of the lung microbiome is determined by three factors: microbial immigration from the mouth and the upper respiratory system, microbial elimination locally, and the relative reproduction rates of its members. In a healthy lung, the microbiome is determined primarily by immigration and elimination; in advanced lung disease which impaired both immigration and elimination, the microbiome is determined primarily by regional growth conditions, and the same species could develop into different lineages. (B) The adapted island model of lung biogeography. Community richness in health for a given site in the respiratory tract is a function of immigration and elimination factors. Speculated positive immigration and negative extinction factors for the lung microbiota are shown. Credit: Similar to Fig. 2 of Dickson RP, Erb-Downward JR, Martinez FJ, Huffnagle GB. The microbiome and the respiratory tract. Annu Rev Physiol 2016;78:481–504. https://doi.org/10.1146/annurev-physiol-021115-105238. Panel A was from Fig. 2 of Stefka AT, Feehley T, Tripathi P, Qiu J, McCoy K, Mazmanian SK, et al. Commensal bacteria protect against food allergen sensitization. Proc Natl Acad Sci U S A 2014;111:13145–150. https://doi.org/10.1073/pnas.1412008111. Panel B was from Fig. 1C,D of Dickson RP, Erb-Downward JR, Huffnagle GB. Towards an ecology of the lung: new conceptual models of pulmonary microbiology and pneumonia pathogenesis. Lancet Respir Med 2014;2:238–46. https://doi.org/10.1016/S2213-2600(14)70028-1.

present in low abundance along with bacteria that may or may not be able to maintain their foothold at the site [90].

In addition to SCFAs and secondary bile acids produced by commensal bacteria [91,92], bacteriophages and anelloviruses might also contribute to the treatment of *Clostridium difficile* (now *Peptoclostridium difficile*) using fecal microbiome transplant (FMT) [93].

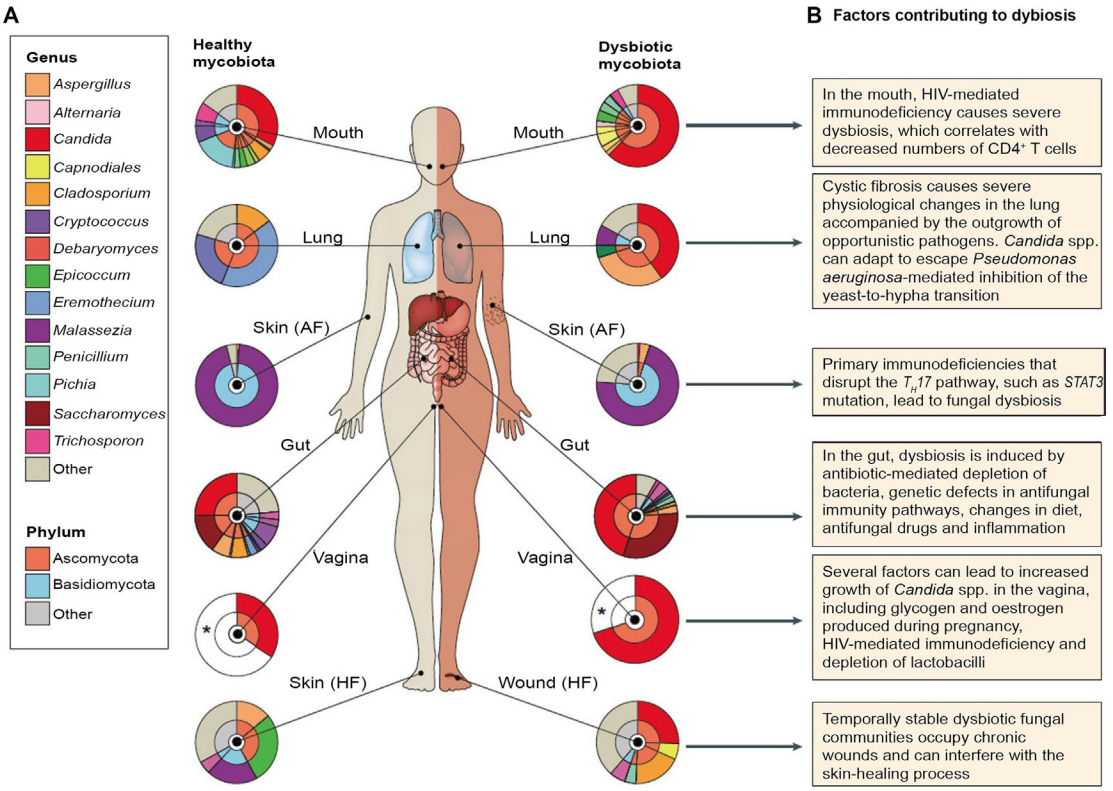

Fig. 4.6 Mycobiota in health and dysbiosis. (A) During homeostasis, diverse fungal communities reside on all human barrier surfaces, such as the mouth, lung, skin, gut, and vagina (left side). The pie charts represent the relative abundance of the observed taxa at the phylum and genus levels (*inner and outer circles*, respectively). Of note, the data for the vagina are estimates that are based on culture-dependent studies, due to a lack of sequencing-based studies related to disease conditions (indicated with an *asterisk*). "Other" refers to sequences with < 5% relative abundance. During disease states, these fungal communities are perturbed (right side). Dysbiotic fungal communities are observed in the oral cavity and the vagina in individuals with HIV; in the lungs of individuals with cystic fibrosis; on the skin of individuals with primary immunodeficiency and chronic wounds; and in the gut of patients with Crohn's disease. (B) Factors contributing to fungal dysbiosis at different barrier surfaces. AF, antecubital fossa; HF, hind foot; STAT3, signal transducer and activator of transcription 3. Credit: Fig. 1 of Iliev ID, Leonardi I. Fungal dysbiosis: immunity and interactions at mucosal barriers. Nat Rev Immunol 2017. https://doi.org/10.1038/nri.2017.55.

4.6 Other omics data that hint at a difference in microbiome

Besides sequencing the microbes themselves, other omics data could also provide very useful information regarding what might have gone abnormal. In animal models such as the pig, metabolites entering and leaving each organ have been systematically studied (Tables 4.2 and 4.3, [94]). Metabolomics technology is also being developed for single-cell measurements [95]. Microbes within each organ, together with the host enzymes, might have contributed to the level of specific metabolites, e.g., amino acids, SCFAs (short-chain fatty acids). These metabolites could further contribute to differential growth or inhibition of microbes [96]. An overarching analogy is that the arteries, veins, and lymphatic circulation are like the sewage system in the 1854 Chlora outbreak in London. It will be important to track down the source of the microbiome culprit.

Table 4.2 Organ-specific metabolite production and consumption in the pig.

Organ	Exemplary discovery	Key evidence
Liver	Clears unsaturated fatty acids	Compared to the most abundant saturated fatty acids, oleate (C18:1) and linoleate (C18:2) show greater uptake and TCA contribution
	Produces amino acids	Significant release of amino acids
(Small) Intestine	Consumes glucose and amino acids	Greatest absolute uptake flux, of any organ, of both glucose and amino acids
Pancreas	Produces TCA intermediates	Significant release of citrate, ketoglutarate, succinate, fumarate, and malate
Spleen	Produces nucleosides	Significant release of cytidine, deoxycytidine, deoxyuridine, guanosine, inosine, thymidine, uridine, and xanthosine
	Produces unsaturated very long chain fatty acids	Significant release of C22:1, C22:2, C22:3, C22:4, C22:5, C22:6, C24:1, C24:2, C24:3, C24:4, and C24:5
Brain	Produces acetate	$> 2 \times$ increase in acetate in jugular vein blood

Continued

Table 4.2 Organ-specific metabolite production and consumption in the pig—cont'd

Organ	Exemplary discovery	Key evidence
Leg muscle	Consumes short-chain acylcarnitines	Significant uptake of C2:0, C3:0, C4:0, C5:0, and C5:1 carnitines
	Produces long chain acylcarnitines	Significant release of C8:0, C10:0, C12:0, C12:1, C14:1, C14:2, C16:0, C16:1, C18:1, C18:2, and C20:4 carnitines
Heart	Consumes long chain fatty acids	Significant uptake of C16:0, C16:1, C18:0, C18:1, C18:2, C20:1, C20:2, C22:4, C24:0, and C24:1
Lung	Produces saturated very long chain fatty acids	Significant release of C22:0 and C24:0
Kidney	Consumes citrate	Only organ with significant citrate uptake; TCA contribution from citrate > 10 × higher than any other organ
	Maintains circulating pyruvate/lactate ratio	Significant increase in pyruvate relative to lactate in renal vein blood
	Produces amino acids	Significant release of amino acids
	Consumes medium and long chain acylcarnitines	significant uptake of C5:0, C6:0, C8:0, C10:0, C10:1, C12:0, C12:1, C14:0, C14:1, C14:2, and C16:1 (without release into urine)

Credit: Table 1 of Jang C, Hui S, Zeng X, Cowan AJ, Wang L, Chen L, et al. Metabolite exchange between mammalian organs quantified in pigs. Cell Metab 2019:1–13. https://doi.org/10.1016/j.cmet.2019.06.002.

Table 4.3 Top three metabolites produced and consumed by each organ in pigs.

	Production		Consumption	
Organ	Metabolite	Log2 (V/A)	Metabolite	Log2 (V/A)
Liver	Glutamate	0.64 ± 0.11	Bile acids (5)	− 2.89 ± 0.19
	Triethanolamine	0.49 ± 0.17	Phenylpropionic acid (2)	− 2.29 ± 0.12
	Acetoacetate	0.38 ± 0.09	Short-chain fatty acids (3)	− 2.02 ± 0.83
Portal (intestine)	Bile acids (6)	3.28 ± 0.21	2-Methylhippuric acid	− 0.69 ± 0.15
	Phenylpropionic acid (2)	2.84 ± 0.32	Glucose	− 0.31 ± 0.05
	Short-chain fatty acids (3)	2.82 ± 1.15	Glutamine	− 0.28 ± 0.02
Colon	Short-chain fatty acids (3)	4.65 ± 1.21	2-Methylhippuric acid	− 0.60 ± 0.21
	Lithocholic acid	4.04 ± 1.10	5-Hydroxylysine	− 0.41 ± 0.05
	Phenylpropionic acid (2)	3.42 ± 0.48	Glucose	− 0.39 ± 0.05
Pancreas	Xanthine	1.05 ± 0.26	5-Hydroxylysine	− 0.79 ± 0.19
	Capryloyl glycine	0.51 ± 0.09	*N*-carbamoylsarcosine	− 0.39 ± 0.09
	TCA intermediates (5)	0.36 ± 0.17	Amino acids (8)	− 0.36 ± 0.01

Table 4.3 Top three metabolites produced and consumed by each organ in pigs—cont'd

	Production		Consumption	
Organ	**Metabolite**	**Log2 (V/A)**	**Metabolite**	**Log2 (V/A)**
Spleen	*O*-phosphorylethanolamine	1.11 ± 0.22	Adenosine	− 0.61 ± 0.14
	Nucleosides (9)	0.52 ± 0.03	Dihydroxymandelic acid	− 0.33 ± 0.07
	C22 and C24 very long-chain fatty acids (11)	0.35 ± 0.008	C5 acylcarnitine	− 0.26 ± 0.02
Head (brain)	Synephrine	1.89 ± 0.68	Dihydroxymandelic acid	− 0.38 ± 0.12
	Gluconolactone and gluconate	1.66 ± 0.03	2-Methylhippuric acid	− 0.34 ± 0.11
	Acetate	1.46 ± 0.39	Glutamate	− 0.30 ± 0.09
Leg (muscle)	Hypotaurine	0.69 ± 0.12	Glutamate	− 1.41 ± 0.33
	Branched chain hydroxyl acids (2)	0.65 ± 0.12	Ketone bodies (2)	− 0.58 ± 0.14
	Medium and long-chain acylcarnitines (11)	0.57 ± 0.02	Short-chain acylcarnitines (5)	− 0.36 ± 0.05
Lung	2-Phenylpropionic acid	0.48 ± 0.14	5-Keto-D-gluconic acid	− 0.31 ± 0.07
	Aconitate	0.26 ± 0.03	Kynurenate	− 0.22 ± 0.03
	C22:0 and C24:0 fatty acids	0.24 ± 0.02	3-Hydroxyanthranilic acid	− 0.17 ± 0.02
Kidney	Glycocyamine	1.87 ± 0.12	*N*-formyl-L-methionine	− 2.66 ± 0.32
	Serine	0.73 ± 0.12	Medium-chain acylcarnitines (4)	− 2.61 ± 0.19
	Allantoate	0.53 ± 0.12	*N*-acetyl amino acids (9)	− 1.27 ± 0.87
Heart	Hypotaurine	0.34 ± 0.11	3-Phenylpropionic acid	− 0.71 ± 0.24
	Glutamate	0.26 ± 0.04	Unsaturated long-chain fatty acids (11)	− 0.53 ± 0.06
	Biotin	0.25 ± 0.06	Hydroxyindoleacetic acid	− 0.47 ± 0.12
Ear (skin)	Guanine	0.82 ± 0.19	Hydroxyhippuric acid	− 0.35 ± 0.08
	Taurine	0.53 ± 0.08	Indole metabolites (2)	− 0.23 ± 0.02
	Long-chain acylcarnitines (3)	0.20 ± 0.04	Serine	− 0.15 ± 0.01

Ranking is based on multiplying the log2 (t value) and log2 (Vein/Artery) to reflect both statistical significance and fold change. Numbers in parentheses refer to the number of metabolites in that category showing statistically significant arterio-venous differences across the indicated organ. All arterio-venous differences included in the table are statistically significant (FDR < 0.05).
Credit: From Table 2 of Jang C, Hui S, Zeng X, Cowan AJ, Wang L, Chen L, et al. Metabolite exchange between mammalian organs quantified in pigs. Cell Metab 2019:1–13. https://doi.org/10.1016/j.cmet.2019.06.002.

Studies on the immune cell populations in each organ are still limited. Hopefully, in the near future, they will be mapped throughout the body, together with the microbiome. Epitopes from engulfed bacteria have been shown to be presented by MHC I in melanoma cells [97] (Fig. 4.7).

For cancer and aging-related diseases, DNA mutation patterns in tissue samples might also suggest the presence of certain microbes [98].

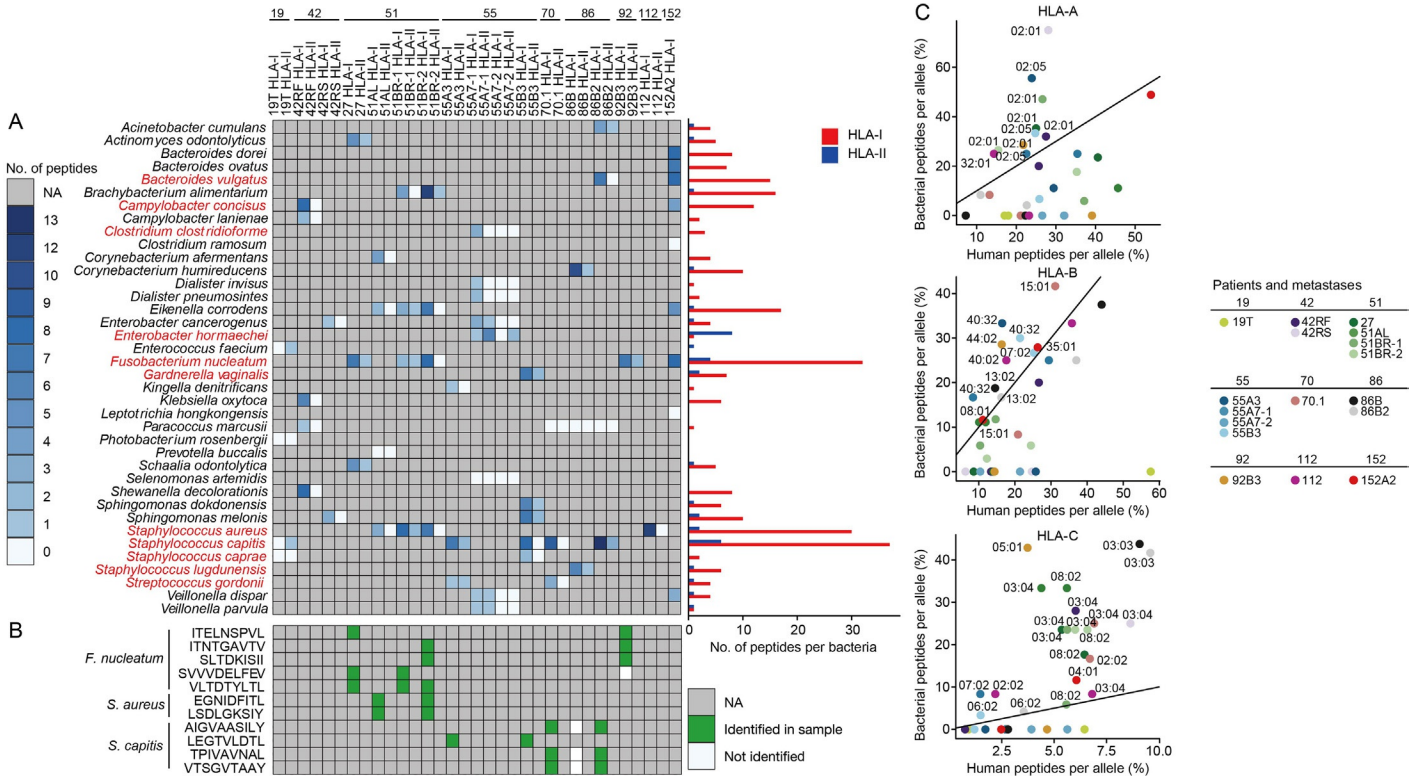

Fig. 4.7 Characteristics of bacterial peptides from melanoma samples. Peptidome was analyzed by mass spectrometry and matched with the proteome of bacteria that were identified by 16S rRNA gene amplicon sequencing, with filtering and validation steps [97]. (A) The number of bacterial peptides presented on HLA-I and HLA-II in each patient sample (patient number indicated at the top) is indicated in a *blue* color scale (left). *White* indicates that no peptides were identified in the sample, and *gray* indicates that the bacterium was not identified in this metastasis (NA, not applicable). The total number of bacterial HLA-I and HLA-II peptides from each bacterium is noted in the bar plot on the right. Species names marked in *red* are known to be intracellular bacteria (Supplementary Table 6) of [97]. (B) Bacterial peptides that were identified in a few metastases from the same patient or in different patients are indicated. Peptides identified in the sample are marked *green*, and *white* denotes peptides that were not identified in the sample (although the metastasis has the required HLA allele for this peptide presentation and the species of bacteria). *Gray* indicates samples that lack the HLA allele and bacteria to produce the peptide. (C) For each metastasis, the percentages of bacterial and human peptides that match each HLA-A (left), HLA-B (middle), or HLA-C (right) allele of the patient is indicated. The allele with the best percent rank binding prediction (by NetMHCpan) was assigned to each peptide; the full allele list is indicated in Extended Data Fig. 6 of [97]. Credit: Fig. 2 of Kalaora S, Nagler A, Nejman D, Alon M, Barbolin C, Barnea E, et al. Identification of bacteria-derived HLA-bound peptides in melanoma. Nature 2021;592:138–43. https://doi.org/10.1038/s41586-021-03368-8.

Worked sample 4.4

After removing a kidney stone, can the patient's fecal, urinary, or oral microbiomes be matched with the type of stone (calcium oxalate (dihydrate, monohydrate, and more complexities [99]), calcium phosphate, struvite, or uric acid stones)? What lifestyle factors shall we inquire about, and can we give the patient some useful advice? (Get ready for Chapters 7 and 8).

4.7 Summary

This chapter focuses on identifying sources of a given microbiome, whether it is from the environment, from members of the family, or from elsewhere in the same person. Knowledge from Chapter 3 will be put into use in all kinds of samples. Many bacteria can enter the lymph nodes or enter circulation. Fungi can lurk in the gut or other mucosal sites before serious symptoms elsewhere. For rheumatoid arthritis, the synovial fluid contained bacterial DNA identified in the oral or fecal microbiome. Many oral microbes have been found in atherosclerotic plaques. The flow of metabolites, and the tissue-resident immune cells, may also provide clues for where to look for the microbial culprits, in order to better understand and treat the diseases (Chapters 6 and 7).

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