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CHAPTER

3

Bacteria, Viruses, and the Microbiome

ROCKING THE BOAT

During the early part of the 19th century, postpartum bacterial infections (puerperal infections) were not uncommon and were fatal in as many as 10–15% of cases. As odd as it may seem from today's standards of care, at the time little was understood regarding bacterial infections and proper hygiene within medical settings. Ignaz Semmelweis had the audacity to suggest that postpartum bacterial infections might stem from unhygienic medical staff or by medical equipment that hadn't been cleaned, and he demonstrated that infection frequency could be diminished by having staff wash their hands with chlorinated lime solutions. These suggestions were not well received (weren't the hands of doctors always clean?). With Lister's discovery of antiseptics in 1865 (published in 1867), thanks in part to Pasteurs work related to "germ theory," the practice of maintaining cleanliness became paramount in surgical practice. Arguably, antiseptics and anaesthetics changed surgery and surgical risks forever. However, poor Semmelweis didn't get to see these breakthroughs in medicine, nor was he rewarded for his observations. Instead he was ostracized from others in his profession. He became progressively more depressed, and his battles with institutionalized medicine eventually landed him in a medical asylum. Upon trying to leave, he was beaten by guards, and died two weeks later as a result of internal injuries or gangrene secondary to his injuries.

The situation within hospitals has obviously improved since then, except that hospital acquired infections have been on the rebound for years, including those that are treatment resistant. One terrible condition that can afflict patients is sepsis, comprising infection that spreads to the bloodstream, and the resulting inflammatory cascade can damage various organ systems, eventually leading to death. Early treatment with antibiotics and lots of fluids had been the treatment of choice, but it would be far better to prevent the condition as Semmelweis had done with regard to postpartum bacterial infections. As it happens, simple procedures, such as increased training for staff and the use of a special observation chart to identify early signs of sepsis, can appreciably reduce its occurrence.

Risk of bacterial infection within hospital settings has gone beyond sepsis. Soon after hospital admission patients often lose commensal gut bacteria, whereas pathogenic bacteria might rise

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(McDonald et al., 2016). This could occur because of the stress of illness, the novel hospital environment, change of sleep pattern, or hospital foods, but whatever the case, this dysbiosis (microbial imbalance) could affect immune functioning, thereby influencing vulnerability to illnesses. With the proviso that resources are available, it may be possible to track individual microbiota that could eventually inform patient vulnerabilities and potentially point to the optimal treatments strategies.

Despite the ignominious treatment Semmelweis received while alive, he was treated better posthumously. He became known as the "saviour of mothers," and the Semmelweis Klinik, a woman's hospital in Vienna, is named after him, as is Semmelweis University in Budapest, and the Semmelweis Hospital in Miskolc (Hungary). The sceptics among us are certainly pleased by the naming of the "Semmelweis reflex," which refers to the reflexive rejection of new knowledge that challenges the old norms and beliefs.

BACTERIAL CHALLENGES

Bacteria, which are thought to be among the first life forms that appeared on earth, are present everywhere, coming in varied sizes and shapes. They typically live in harmony with plants and animals, acting symbiotically, but can also function in a parasitic relationship with other living things. Many of the trillions of bacteria present in animals (and humans) have important beneficial actions, whereas others are less kind, causing a variety of diseases. Under the right environmental conditions, including the temperature and pH, availability of water, oxygen, and a source of energy, bacteria will grow to a particular size and then reproduce asexually through binary fission. Bacteria can double in number every 30 minutes, and some bacteria can do this within 10 minutes. Certain bacteria, such as streptococcus, can stay alive for some time on various external objects (e.g., door handles, toys, and cribs) and thus can represent a fairly persistent threat. Other bacteria, like their viral cousins, are subject to airborne transmission, droplet contact, or direct physical contact. It seems that different strains within the same bacterial species can engender very different immune responses, which may contribute to the diverse outcomes elicited across individuals (Sela, Euler, Correa da Rosa, & Fischetti, 2018).

Infection stemming from bacteria and viruses can also be transmitted indirectly. For example, carrying an infection on unwashed hands, depositing these on a surface, which is then touched by another person, can lead to infection being passed along (fecal-oral transmission). As we know from a number of other illnesses, such as cholera, dysentery, diphtheria, scarlet fever, tuberculosis, typhoid fever, and viral hepatitis, disease agents can also be transmitted through water, ice, food, serum, plasma, or other biological products. In some instances, a disease (e.g., the bacterial infection syphilis, or the parasitic disease toxoplasmosis, as well as viruses, such as HIV and measles) can be passed from a pregnant mother to her fetus. Furthermore, zoonotic diseases in which infection is transmitted from animals to humans, are a constant threat, but can become exceptionally hazardous if they mutate so that they can then be passed between humans.

As we'll see in ensuing chapters, viruses and bacteria, by virtue of inflammatory processes being activated, and the downstream

actions on many hormones, brain neurotransmitters, and growth factors, may contribute to several psychological disturbances as well as a great number of physical disorders. To a significant extent, common mechanisms account for these varied conditions, and may be responsible for the frequent comorbidities that are seen amongst illnesses. Increasingly, the significance of infection in relation to mental illnesses has been acknowledged. Methods to prevent or control infection are thus essential in this capacity, but as we'll see, the very best treatments to ameliorate bacterial infection may destroy commensal gut bacteria, leading to the emergence or exacerbation of other illnesses. Clearly, the sword cuts both ways.

Antibiotics

The development of penicillin, and other antibiotics to fight bacteria, was undoubtedly among the most important medical discoveries of the first half of the 20th century. Although Alexander Fleming, who identified penicillin obtained from particular molds, is usually given the credit for antibiotics, infections have been treated with mold extracts for about 2,000 years. In general, antibiotics either kill bacteria (being bacteriocidal) or inhibit their multiplication (bacteriostatic). They do this by either preventing bacteria from building cell walls (e.g., by affecting bacterial ribosomes involved in the creation of cell walls), or breaking down the cell walls of bacteria that already exist. Some antibiotics, such as quinolones, disturb DNA and prevent their repair, so that the bacteria are unable to reproduce and thus die off. Based on the response to a gram stain, and characteristics of the cell walls, bacteria are designated as either gram-positive or gram-negative (the latter being more resistant to antibiotics). When the nature of the bacterial infection is known, a narrow spectrum antibiotic is used, whereas a bacteria that has not been identified is treated with a broad spectrum antibiotic. The former is preferable as they are less likely to create antibiotic resistance. Some antibiotics can produce uncomfortable side effects, and in some instances, allergic reactions can occur that cause anaphylaxis.

Antibiotic Resistance

We grew accustomed to being able to destroy bacteria through treatment with antibiotics, and for some time it had simply been assumed that when one antibiotic failed to do the job effectively, then another could do the trick. Ironically, their very effectiveness contributed to their undoing. As bacteria began to form resistance to antibiotics (reflected by greater difficulty in treating some infectious diseases, lengthier recovery times from infection, and the probability of death increasing), and the first alarms were sounded, a generally cavalier attitude persisted, and most people continued to behave as they had previously. Inevitably, most bacteria followed an effective game plan to get around antibiotics, and hence they all successively became less effective or entirely ineffective.

The factors that generated treatment resistant bacteria comprised the perfect storm. One should never have imagined that bacteria were passive travelers who were simply waiting to be killed by antibiotic agents. Instead, like an opposing army (or groups of terrorists) bent on the host's destruction, some harmful bacteria are clever and vicious, so that with time and experience they develop resistance to the drugs. It was suggested that in response to stressors, such as nutrient deprivation, microbiota respond in a coordinated manner to deal with the insult. Being a new challenge for bacterial communities, an antibiotic might result in bacteria rapidly searching for new methods of dealing with the challenge. Ultimately through a process much like natural selection based on random mutations occurring, bacteria

develop resistance to the antibiotic (Jensen, Zhu, & van Opijnen, 2017).

The ability of bacteria to become resistant might have been facilitated by the inappropriate use of antibiotics to fight viruses (e.g., strep throat, bronchitis) for which antibiotics are ineffective. In fact, in the face of a serious threat, such as an antibiotic treatment, bacterial mutation rates increase appreciably, thereby increasing the probability of a mutation occurring that will protect the bacteria from destruction. Furthermore, it was thought that when confronted with an antibiotic, especially if the full course of treatment wasn't adopted (because patients felt better and believed they no longer need the antibiotic, or because they were saving pills in the event that they were needed at some later time), a few hardy bacteria may survive. This may then give rise to similarly resistant clones, so that over successive generations and increased development of evasion methods, the effectiveness of antibiotic agents diminishes[1].

The rate of bacterial mutation increases with a person's age as well as with the social environment in which bacteria find themselves. At the other end of the age spectrum, babies born very prematurely are at increased risk of illnesses developing. As a matter of course, preemies were treated with antibiotics in the mistaken belief that this couldn't cause harm, but this resulted in a marked decline in the diversity of microbiota and simultaneously enhanced survival of bacteria that are resistant to antibiotics. Thus, should a blood infection subsequently arise, a large proportion of these infants will not fair well, especially as resistance to one type of antibiotic also dials up resistance to other antibiotics (Gibson et al., 2016).

The massive use of antibiotics in animals to prevent them from developing infections has contributed to resistant bacteria evolving (Johnson et al., 2016). The antibiotic infested meats end up on our dinner plates, and thus contribute to the development of resistance. As well, some rivers and streams contain *Escherichia coli* resistant to antibiotics. There's a good chance that animal waste, laden with antibiotics and antibiotic-resistant bacteria, leeched into waterways. Air pollution may also affect commensal bacteria, and influence resistance of some bacteria (*Staphylococcus aureas* and *Streptococcus pneumonia*) to antibiotic treatments (Hussey et al., 2017). There has also been the reasonable concern that some household products, such as the disinfectant triclosan, may contribute to antibiotic resistance. As a result, it has been banned from hygiene products, such as hand, skin, and body washes, but triclosan and similar agents appear in numerous other products.

In addition to the mutations that are due to overuse of antibiotics, bacteria have several dirty tricks that they can fall back on. For instance, the genes involved in the development of resistance can be transferred to other cells (conjugation) so that they too will become resistant, although it may be possible to prevent or reverse this action (Lopatkin et al., 2017). Furthermore, in response to an antibiotic, bacteria can go dormant, making them less likely to be attacked (termed persistence). With repeated antibiotic attacks, they essentially "learn" to stay in the dormant state for periods that line-up with the antibiotic's actions, emerging once it seems safe (Fridman, Goldberg, Ronin, Shoresh, & Balaban, 2014). On top of this, bacteria seem to act collectively, coordinating their actions to render maximal toxic

[1] The seemingly common sense perspective concerning overuse of antibiotics was almost universally accepted, even though it has been argued that there was actually little evidence supporting this contention. Physicians may be loath to undertreat patients, and thus typically prescribe based on precedent, which could actually reflect *overtreatment*, thereby placing patients at increased risk for antibiotic resistance (Llewelyn et al., 2017). Rather than destroying bacteria entirely, it might be sufficient to simply slow down potentially dangerous bacteria, and in doing so it is less likely that antibiotic resistance will develop (Spellberg, 2016).

effects based on messageing from some external source (quorum sensing), such as the medium in which the bacteria are present (Ng & Bassler, 2009). Bacterial communities can secrete substances, such as β-lactamase, which can proffer passive resistance for other bacteria that are present in that particular environment. Similarly, bacteria that express the resistance factor chloramphenicol acetyltransferase (CAT) can deactivate antibiotics present in that immediate environment. In essence, the response to an antibiotic could be affected by the microbial environment that is present (Sorg et al., 2016).

ANTIBIOTIC RESISTANCE AS AN INCREASING WORLDWIDE THREAT

The WHO has indicated that antibiotic resistance has become among the most pronounced threats to global health and food security. Several bacterial species can cause illnesses by acting as "opportunistic" pathogens. These common threats comprise species such as E. coli and those referred to as the ESKAPE organisms, comprising Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter, Pseudomonas, and Enterobacter. Several species and strains of commensal bacteria will readily be destroyed by antibiotics, but may ultimately be replaced by those that are resistant. One of the more recent threats has come out of China, where a hypervirulent form of K. pneumoniae emerged that was multidrug resistant, as well as highly transmissable (Gu et al., 2018).

Staphylococcus aureus (S. aureus or Staph infection), is the best known and most frequent cause of postsurgical infection, but hospital acquired infections have also included bacteremia (bacteria in the blood), endocarditis (inflammation of the inner layer of the heart), sepsis, toxic shock syndrome, meningitis, and pneumonia. We had counted on antibiotics, such as methicillin, in the treatment of such conditions, but a bacterial strain evolved that stopped responding to this agent. It has been estimated that hospital-acquired infections within the US, particularly methicillin-resistant S. aureus (MRSA) and Clostridium difficile (C. difficile) doubled between 2000 and 2010. These infections occurred in about two million patients, leading to between 23,000 and more than 100,000 deaths yearly. Following a hospital stay, one of four seniors had antibiotic resistant bugs on their hands, which they could spread elsewhere (Cao, Min, Lansing, Foxman, & Mody, 2016). Likewise, antibiotic resistance is relatively more common among diabetics using insulin, individuals undergoing chemotherapy, or who have burns, cuts or lesions on the skin, patients undergoing breathing intubation, or who have urinary or dialysis catheters inserted, as well as those with HIV/AIDS or with a weakened immune system owing to still other factors. The immunosuppressive actions of stressors can likewise increase vulnerability to S. aureus infection, especially in vulnerable populations, such as older people.

It is worrisome that MSRA has surfaced outside of hospital environs, becoming a community-acquired infection. It has become increasingly common within individual homes, infecting meat, and poultry. Community-linked infection recently stood at 12%, being attributed to sharing contaminated items, active skin diseases or injuries, poor hygiene, and crowded living conditions. Furthermore, antibiotic resistant bacteria can be "picked up" from other people or foods, as observed among tourists who visit countries where these bacteria are relatively common. The good news, even though it's still a bit limited, is that analyzing the DNA of MSRA can identify those individuals who are at greatest risk of dying as a result of infection, and could potentially facilitate the development of personalized treatment strategies (Recker et al., 2017).

Some illnesses that we hadn't thought about becoming resistant to antibiotics are doing just this. Neisseria gonorrhea, which is responsible for gonorrhea has been showing increased signs of resistance to antibiotics, no longer being responsive to some agents (Unemo et al., 2017). Quinolones, a class of antibiotics that had long been used to treat gonorrhea, have lost their effectiveness, and other drug classes, such as cephalosporins, have also been losing their effectiveness. The last line of defense, the go-to antibiotic colistin, was recently found to have lost its effectiveness in certain cases, possibly owing to a transferable gene mcr-1 that makes it resistant to colistin. This gene can appear in a variety of bacteria and consequently they too could potentially develop resistance.

It's only a matter of time before other threats emerge for which we have little protection or cure. One of these, Shigella currently affects upward of 165 million people worldwide, most often being transmitted through the "the fecal-oral" route. Historically, this highly contagious condition was treated successfully with ciprofloxacin, but its efficacy is now questionable (CDC, 2016). Further, some bacterial illnesses that should have been wiped out years ago, such as tuberculosis (TB), still haunt us. While largely eliminated in Western countries, it is still devastating within parts of Asia and Sub-Saharan Africa, infecting about 9−10 million people in 2015, leading to about 1.5 million deaths, and more than 600,000 individuals have a treatment resistant form of the illness. An antibiotic-resistant form of typhoid has also evolved, infecting large numbers of people within Asia. As typhoid infections ordinarily occur in as many as 30 million people each year, the spread of a treatment resistant strain may be devastating to an already illness-ridden population. As much as basic health conditions are required to beat various diseases, the development of treatment resistant bacteria, together with the lack of funds or global political will, may limit prevention and treatment of illnesses (WHO, 2016a,b).

Dealing With Antibiotic Resistance

As a first step to combat antibiotic resistance, it might be appropriate to limit the use of these agents for minor bacterial infections. Failing this, alternating doses of antibiotics, and changing the particular antibiotics administered with successive infections might be helpful. Combinations involving several antibiotics administered concurrently that can act synergistically have shown promise in dealing with particular bacteria, and the use of two compounds, one that shreds the shell of bacteria, and the second a potent antibiotic that attacks the exposed bacteria, may be useful in dealing with resistant bacteria (Stokes et al., 2017). As the development of antibiotic resistance has been attributed to the ability of bacteria to limit antibiotic entry into cells, as well as the production of an enzyme, β-lactamase, which is able to destroy antibiotics, β-lactamase inhibitors have been developed to attenuate resistance (Jiménez-Castellanos et al., 2018).

It is also possible to act on bacterial genes to make them more sensitive to antibiotics. A novel compound Teixobactin, which was isolated from microorganisms present in soil, was capable of destroying pathogens effectively, including C. difficile, septicemia, and tuberculosis, without resistance developing. Teixobactin seemed to be effective because it attacks bacteria through multiple methods,

and resistance to its effects may not be seen for several decades (Ling, 2015). The discovery of Teixobactin effectiveness was soon followed by several analogs of this compound, and there is a good chance that this is a first step in the development of new antibiotics. Even with so many new treatment in the works to deal with bacterial infection, given the ruthlessness of bacteria in finding ways around our treatments, there is the concern that these treatments will meet the same fate as the antibiotics that are currently available.

Alternatives to Standard Antibiotic Treatments

Severeal novel approaches have been used to eliminate bacteria. Efforts have been directed to treat specific conditions by having bactria turn on one another. Bacteriocins (proteins produced by bacteria to kill their own competitors) could be harnessed to kill pathogens while leaving other microbiota intact. It similarly appears that particular strains of C. difficile are adept at destroying each other (they are competitive strains) by firing a harpoon-like needle through their membrane, which promotes the death of the cell. Thus, the interesting notion was broached that the human microbiota could be used as a potential source for the development of novel ways of dealing with bacteria (Kirk et al., 2017). Using a somewhat different approach, it was demonstrated that resistant bacteria, such as MRSA, could be manipulated by altering ingredients that they need for survival. For instance, MRSA is reliant on folate (vitamin B9), and hence blocking the production of folate can be used as a way of overcoming their resistance to treatments (Reeve et al., 2016).

Viruses have been identified that attack bacteria (termed bacteriophages, or simply phages). These phages appear in mucus, such as in the gums and gut, and may influence immune functioning. The development of viruses that can deal with resistant bacteria, such as MRSA (Green et al., 2017), would be welcome, but it's still very early and the extent to which phages can be used in this capacity isn't altogether certain. In addition to these approaches, nanoparticles have also been developed that can produce chemicals effective in destroying otherwise treatment resistant bacteria (Courtney et al., 2017) and CRISPR-Cas9 could potentially be used to cut out genes from bacteria that show resistance to antibiotic treatments. Appreciable attention has also focused on developing strategies, as well as computer algorithms, that would inform best treatment approaches (Bucci et al., 2016).

VIRAL ILLNESSES

Several viruses, like their bacterial cousins, may contribute to illnesses that have psychological ramifications, which we'll consider in later chapters. Viruses are often said not to be a life form since they are not able to reproduce unless they have the opportunity to use a cells machinery to do so. Upon penetrating a cell, the virus enters into the host cell's genome, and thus uses it in order to replicate. Once sufficient replication has occurred, the virus can force itself through the host cell's membrane, and the viruses that escape will have the opportunity to infect nearby cells. As the virus has its own complement of genes, they can mutate so that new variants of the virus can appear.

Viruses can spread from one person to another through various routes (e.g., through the air or through body fluids), and they can linger for various amounts of time within external environments. In some instances, a

virus can lie dormant within the body for extended periods before re-emerging to induce an illness. Viruses and bacteria can also be transmitted to humans through a vector, such as mosquitoes or ticks, leading to illnesses such as malaria, Zika, West Nile virus, dengue fever, and yellow fever, and severe illnesses have spread to humans through birds, pigs, cattle, and rodents. Typically, vector-borne viruses typically don't make the leap to being transmitted between humans. However, these viruses can mutate, and could potentially be transmitted between humans, leading to diseases such as swine flu, HIV/AIDS as well Ebola.

The virulence of a microbe varies so that some create mild symptoms, whereas others can have rapid and powerful consequences. How quickly and broadly a virus can spread within a human population is dependent on several factors; (1) how readily it can be passed one from one person to another, (2) the route by which it is transmitted (e.g., aerobic transmission is more rapid than transmission that involves exchange of fluids), (3) the ability of the virus to penetrate the host's tissues and enter into cells, (4) the capacity of the virus to inhibit the host's immune defenses, and (5) how well equipped it is in obtaining nutrition from the host. Although it is often thought that transmission varies with how quickly the virus kills the host, given that death of the infected person diminishes the opportunity for viral transmission, although passage from one person to the next, as in the case of Ebola, may come about even after death.

In some cases, viruses have nefarious ways of getting around the host's immune defenses. Using particular proteins, they can mask themselves so that they are not readily recognized by immune cells (Holm et al., 2016), and with the assistance of other proteins (neuraminidase), as in the case of influenza virus, for instance, they are able to counter the attack of NK cells that would otherwise destroy the virus. Fortunately, inhibitors of neuraminidase have been developed that enhance the effectiveness of NK cells, and antibodies have been created that act act against proteins that limit NK cell activity (Bar-On, Seidel, Tsukerman, Mandelboim, & Mandelboim, 2014).

PEOPLE NOT TO HANG WITH

People react differently to viruses and to vaccines. Women, in general, seem to be more reactive to vaccinations, possibly because of hormonal factors increasing immune activity. As well, a protein TLR7 which detects viruses and effectively activates immune cells is encoded by genes present on the X chromosome, and hence leads to a greater immune response among women than in men (Karnam et al., 2012). While the greater immune responses among women might seem advantageous, it could also contribute to the greater female disposition toward autoimmune disorders.

Some individuals, often referred to as "superspreaders," seem to be particularly adept in passing on viruses and bacteria. Some feature of their immune response might be responsible for this facility, or they may may have occupations that lead to more social contact either directly or indirectly, or they may simply be especially social, thus coming into contact with a particularly large number of people. Mary Mallon, a cook in the early 1900s seemed to have been a virtuoso in spreading typhoid, despite not presenting with any symptoms herself. She is now best known as "Typhoid Mary" for having infected 51 people, several of whom died. Today's version of Mary Mallon would be far more dangerous owing to larger populations, crowded conditions, and more efficient travel. Indeed, the Middle East Respiratory Syndrome (MERS) that affected South Korea from May to July of 2015, infected 186 individuals, of which 36 died, and caused quarantine of thousands

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more. It turns out that an individual who contracted the illness transmitted it to another person, who then reported to hospital with respiratory difficulties. But, as MERS wasn't yet on the radar, he wasn't isolated, and over the course of the next few days he infected 82 others, accounting for 45% of the cases during the outbreak. The Pareto principle, also known as the 80–20 rule, seems to be pertinent to the spread of infection in that 80% of cases transmitted occur through 20% of the people. We can only hope that the potential spreaders choose to be vaccinated, but failing this, we might get lucky and they'll find friends other than us.

Vaccines

For centuries viral illnesses (as well as bacterial infections) decimated human populations, but the discovery of vaccines to prevent illnesses was an obvious game-changer. Using inactivated or dead virus the immune system is primed to respond to similar viruses when they are encountered subsequently, thereby preventing the illness from occurring. Despite the effectiveness of many vaccines, others have been less than perfect, varying across individuals and in some instances their effectiveness diminishes with age. Viruses are also able to mutate so that they won't be recognized. Influenza viruses are notorious in this regard, but since they come in a set number of formats, vaccine makers may (often) be able to anticipate next year's threats. Still, the accuracy of these predictions are variable, such that in some years the vaccine created will be very good, but in other years it has almost no effect. Even if the vaccine is an effective one, individuals vary in the extent to which they are "vaccine responders," possibly owing to whether they produce sufficient antibodies to fight future infection. As well, some flu virus mutations tend to be preferentially effective in infecting immune-compromised individuals. These individuals might be especially sensitive, and they may serve as harbingers of the virus mutations that will be evident during next year's flu season (Xue et al., 2017).

Some vaccines can be developed readily (as in the case of many seasonal flu vaccines, although effective immunization runs around 50–60%), but developing others are more difficult owing to rapid mutations that occurred, as in the case of H7N9 bird flu. This virus spreads from birds to humans and hopefully won't mutate so that the virus spreads between people. However, the CDC has ranked H7N9 at the top of the list of flu strains that could produce a human pandemic, making it essential that new vaccines be available.

It's thanks to mass vaccinations that diseases such as polio have been almost eradicated and measles, mumps, and rubella, which also caused many deaths, have been diminished. Because it takes some time for vaccines to be produced, even when the correct vaccine has been identified, methods are being developed that might be made more rapidly. "Naked DNA" vaccination is one possible approach to this. Administering a viral gene (as opposed to the virus itself) into animals is known to elicit an immune response. Once sections of DNA that encode a viral gene are injected, nearby cells take up the DNA, and will form proteins associated with the virus, to which the immune system ought to mount a response and like other methods of vaccination, a memory of the virus should be maintained.

Given the moderate efficacy of current vaccines, it might be fruitful to develop new

approaches to enhance their effectiveness. It has been maintained that gut microbiota may have regulatory actions in relation to influenza, and thus could potentially be harnessed to limit the consequences of influenza infection (Chen , Wu, Kuo, & Shih, 2017). There have been efforts to develop peptide molecules that are able to inhibit a variety of influenza strains by grasping onto common features of a set of influenza A viruses (Kadam, Juraszek, Brandenburg, Buyck, & Schepens, 2017). At some time, a universal vaccine will be developed that acts across a still broader range of influenza viruses (Paules, Marston, Eisinger, Baltimore, & Fauci, 2017).

Just as some individuals may be virus superspreaders, there seem to be those who are particularly susceptible to infection. In this regard, individuals who choose not to be vaccinated (or have their children vaccinated) leave themselves open to illnesses. There are many reasons (or rationalizations) for individuals choosing not to be vaccinated for common illnesses. Frequently, there is mistrust of media and government agencies with respect to recommendations that have been made concerning vaccination (Taha, Matheson, & Anisman, 2014). Alternatively, they may be listening to the sage advice coming from a large cadre of Hollywood types, a few politicians, or friends with strong, albeit fallacious opinions, about the possibility that vaccination is dangerous.

A detailed analysis pointed to a fairly extensive set of factors that were linked to individuals choosing whether or not to be vaccinated (Nowak, Sheedy, Bursey, Smith, & Basket, 2015). Those who opted not to be vaccinated may have based their decisions on earlier experiences, such as having had a negative response to a vaccine, or beliefs that the illness (e.g., flu) is manageable. Resistance to vaccination is also attributable to the belief that recommendations for vaccination actually might be correct for others, but don't apply to them. Some individuals believe that vaccines are often ineffective, or the the misguided notion that one could get the flu (or another illness) from a vaccine. Those railing against vaccinating their children might also not have had the experience of growing up at a time when illnesses like measles, were damageing or killing children, and diseases such as polio were a horrible threat that kept reappearing[2].

Predictably, those ammenable to receiving (flu) vaccination tended to believe that they were flu susceptible, and that vaccines were effective. The propensity to be vaccinated was also elevated among older people or those having an existent chronic health conditions that might be complicated by becoming ill. Having previously experienced a bad flu or a similar illness, favored individual's choosing to be vaccinated, as did easy access to vaccination. As well, intention to vaccinate was particularly high if the recommendation to do so came from a physician[3].

Some viruses, such as measles, are remarkably effective in spreading, so that one person might infect about 90% of people close to them. Other viruses spread less readily, so that one person may infect very few others (say, 0.5

[2] In considering the factors associated with depressive disorders (Chapter 8), the work of Kahneman and Tversky related to decision making was raised in the context of how individuals appraise stressful events. Their work indicated that individuals are apt to make some seemingly puzzling decisions in certain situations, and may have much to say about the irrational decision making processes that are common in relation to whether or not people choose to be vaccinated.

[3] In some studies, participants are asked about their "intent" to be vaccinated. While this is reasonable, intent doesn't necessarily translate into action (i.e., actually being vaccinated), and so the data must be interpreted caustiously. It is certainly of interest to determine what factors determine whether intentions become actions.

people), and thus the disease will disappear. Fortunately for individuals who choose not to be vaccinated, when enough people in a population are vaccinated, the source for transmission will be diminished, and thus even potent viruses might not spread (herd immunity). Ironically, this herd immunity also protects the children whose parents refused to have them vaccinated. However, should antivaxxers be successful in their campaign, so that enough people within a population decide not to be vaccinated (or not have their children vaccinated), a "tipping point" will be reached so that herd immunity no longer protects people who are not vaccinated, or those in whom the inoculation was not particularly effective (i.e., vaccine nonresponders). Should an individual become infected with measles (including the children whose parents decided against having them vaccinated), they will have a tough illness to deal with, and they may also experience serious downstream effects. Specifically, following measles infection the immune system may be altered, possibly for as long as 2–3 years, so that the risk for other illnesses may be elevated (Mina, Metcalf, de Swart, Osterhaus, & Grenfell, 2015). Furthermore, if the immune system is not fully developed, as in the case of young children, infection with measles may result in the virus hiding in the body, only to emerge years later to infect the brain.

Bacterial and Viral Challenges Affect Hormonal and CNS Processes

Pathogenic stimuli, such as bacteria and viruses, cause marked effects on glucocorticoids and on central neurotransmitters. Many of these changes are comparable to those usually elicited by both psychological and physical stressors, and thus it was suggested that these systemic challenges were interpreted by the brain as if they were stressors (Anisman & Merali, 2002). In addition to affecting brain neurotransmitters, such as norepinephrine and serotonin (Hayley, Lacosta, Merali, van Rooijen, & Anisman, 2014), immune activating agents may influence the presence of growth factors (e.g., BDNF) as well as proinflammatory cytokines, presumably released by microglia (Audet & Anisman, 2013). As expected, these outcomes vary with sex and age, and at least some of the effects of immune challenge are subject to a sensitization-like effect in that exaggerated responses are evident upon reexposure to a challenge. Moreover, bacterial agents and stressors may act cooperatively in producing brain neurochemical changes that favor the development of depressive disorders (Anisman, 2009).

As described in Chapter 2, The Immune System: An Overview, multidirectional communication occurs between immune, autonomic, microbial, hormonal, neurotransmitter, neurotrophin, and other brain-related processes. These systems are so intimately intertwined that actions in any one may influence the functioning of others. By example, when mature lymphocytes are not present, ordinary behavioral stress responses might be absent, even in mice that are very stress sensitive (Clark et al., 2014). Likewise, manipulating microbial processes may come to influence brain functioning tied to mental health.

MICROBIOTA

For a time, limited attention had been devoted to the links between the brain and the enteric nervous system, other than analyses related to hunger and satiety. In fact, it came as a bit of a surprise that the brain influences gut functioning, and that signaling through processes that line the esophagus, stomach, small intestine, and colon, affect brain functioning (Bravo et al., 2012). Messages from the gut to the brain may occur through stimulation of the vagus nerve, which extends from

the viscera to the brain stem, and gut processes can influence hormones, such as ghrelin, that affect brain activity, thereby moderating hunger and obesity. As well, gut functioning may influence immune processes (Hooper, 2012), and by virtue of effects on brain functioning, may influence mood and reward processes (Mayer, Knight, Mazmanian, Cryan, & Tillisch, 2014).

Although most of the research concerning microbiota have focused on those residing within the gut, bacteria that affect us are also found in the mouth and nasal passages, on our skin, between our teeth, and within other body orifices. The trillions of bacteria present ordinarily live harmoniously with one another (i.e., commensal bacteria). Over the course of evolution various microbes adapted and colonized different parts of the body. For their mutual benefit many bacteria behaved cooperatively, although others were parasitic, consuming other bacteria (Silverman et al., 2017). In general, when an imbalance occurs between "good" and "bad" microorganisms (dysbiosis) or in the absence of specific types of bacteria being present, an immense range of physical and mental illnesses may follow. Over the short run, animals with a compromised microbiome can survive, but their ability to do so will be curtailed owing to disturbed immunological functioning. Rodents born entirely germ-free have their immune development hindered, and the balance between various aspects of the immune system is disturbed, thus rendering them more vulnerable to pathologies. Even the response to vaccines, known to be highly variable across individuals, may be dependent on the gut microbiome, which contributes to the shaping of immune responses (Lynn & Pulendran, 2017).

Gut bacteria, in the main, come from four phyla, Bacteroidetes, and Firmicutes, Actinobacteria, and Proteobacteria, whereas others, such as Fusobacteria and Verrucomicrobia phyla appear in lesser abundance. As depicted in Fig. 3.1, gut bacteria and their metabolites can affect immune, neurotransmitter, and hormone systems, and may thus influence inflammatory diseases, neurodevelopmental disorders (e.g., ADHD, autism spectrum disorder),and several mental illnesses (Hyland & Cryan, 2016; Schroeder & Bäckhed, 2016), and may even affect sensitivity to cocaine reward and thus increase the risk for addiction (Kiraly et al., 2016).

What we eat influences our gut microbiota (David et al., 2014), and gut microbiota can influence what we eat. Gut bacteria help to break down foods and contribute to the absorption of otherwise difficult to digest substances. Thus, their presence may help individuals stay lean, and many useful bacteria are themselves strengthened by fiber-rich foods. When fiber isn't available, microbes may die off, or they may feed on the mucus lining, which ordinarily keeps the gut wall healthy. Some foods, such as modest amounts of wine, increase the presence of *Pediococcus pentosaceus* CIAL-86, which sticks to the intestinal wall and fights against bad bacteria (Garcia-Ruiz et al., 2014). Other foods, such as emulsifiers (food additives that serve to stabilize processed foods) can have negative health consequences, possibly by altering the gut microbiota and the induction of inflammation, and these effects may be linked to particular genes (Chassaing et al., 2015).

Given their potential health benefits, gut bacteria have become a target to help individuals deal with obesity. For instance, diets spiked with modified bacteria diminished eating and altered metabolism, thereby lowering adiposity and insulin resistance (Chen et al., 2014). As well, some bacterial families, such as Christensenellaceae, appear in greater number among thin individuals than in people who are heavy, and may causally contribute to this difference. When the bacteria associated with slimness were transferred to other mice, weight gain was diminished relative to mice that hadn't received this transplantation. In theory, Christensenellaceae could be useful in

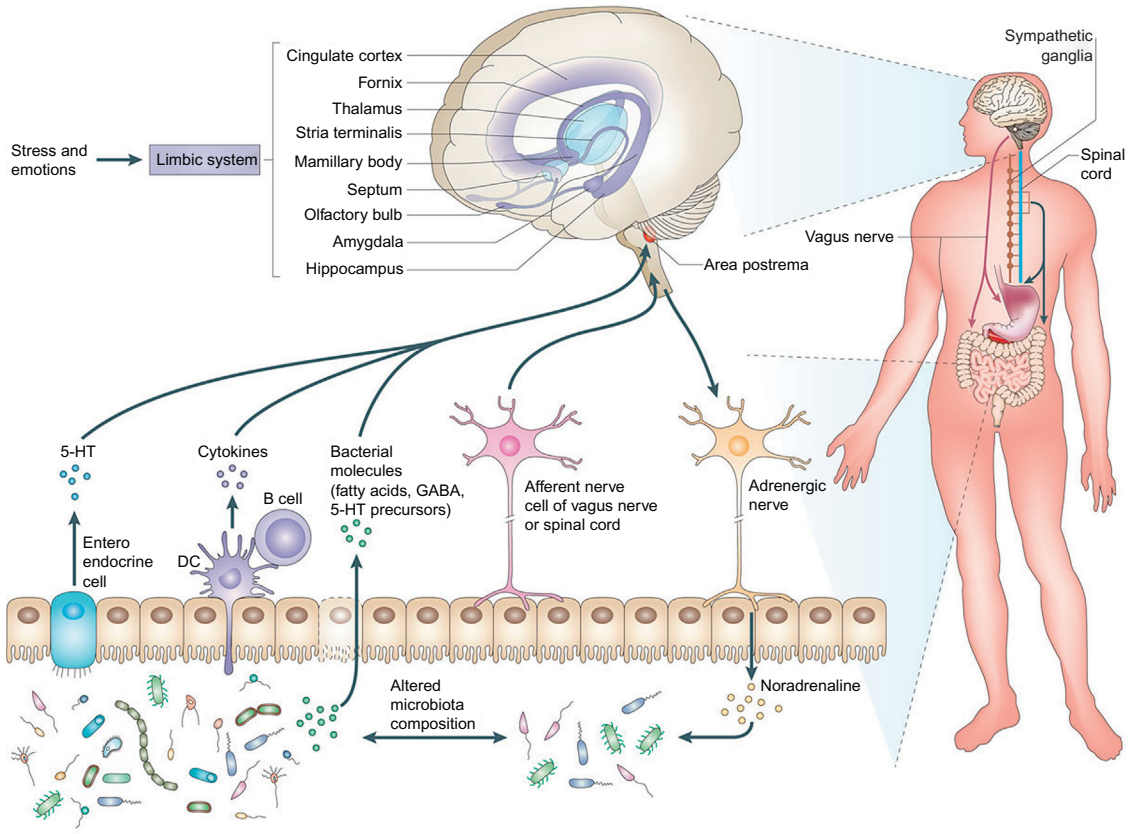

FIGURE 3.1 Neural, immunological, endocrine, and metabolic pathways by which microbiota influence the brain, and the proposed brain-to-microbiota component of this axis. Bacteria and bacterial products can reach the brain via the bloodstream and gain passage to the brain though the area postrema. Commensal bacteria may form ligands that activate G-protein coupled receptors (GPCRs), which are fundamental signaling pathways for a majority of neurotransmitters (Cohen et al., 2017). Brain functioning can also be affected by cytokine release from mucosal immune cells, as well as through the release of gut hormones, such as serotonin (5-HT) from enteroendocrine cells, or via afferent neural pathways, including the vagus nerve. Stress and emotions can influence the microbial composition of the gut through the release of stress hormones or sympathetic neurotransmitters that influence gut physiology and alter the microbiota habitat. Moreover, host stress hormones, such as norepinephrine, might influence bacterial gene expression or signaling between bacteria, which can alter the microbial composition and activity. Immune elements, such as antibody (IgM) secreting B cells, also contribute to microbial diversity (Magri et al., 2017). As multidirectional communication occurs between gut bacteria and immune, autonomic and brain processes, factors that affect brain neuronal activity may influence the microbiome and immune functioning. DC, dendritic cell; GABA, γ-aminobutyric acid. *Source: From figure and portions of the text are from Collins, Surette, & Bercik (2012).*

reducing weight, but it's still early to assess this in humans.

The link between the microbiome and obesity is complex and involves multiple steps, and could be subject to genetic differences across individuals (Duranti, Ferrario, van Sinderen, Ventura, & Turroni, 2017). Gut bacteria also differ between males and females, and

may be differentially affected by diet. These sex differences may be related to hormonal factors (estrogen, in particular) interacting with gut microbes. It is conceivable that diet in men and women will also have different effects on illnesses related to bacteria, and thus diets meant to treat particular disturbances need to be tailored on the basis of sex as well as other individual difference factors that span several domains.

In retrospect, it isn't surprising that gut bacteria play a prominent role in feeding and energy processes, and may contribute to eating- and gut-related disorders (e.g., Bäckhed et al., 2015; Dinan & Cryan, 2016). In an effort to assess the contribution of the microbiota to a variety of phenotypes, analyses were undertaken to assess mice born and raised in germ-free environments. These mice seemed not to develop in the usual fashion, in that their immune system was deficient, and the gut of these mice had a smaller surface area and hence could not absorb nutrients as readily as in mice raised in a standard germy environment. The germ-free mice also had leaky intestinal walls, and blood vessels that ordinarily supplied food to the gut wall were in short supply. Upon receiving microbiota harvested from the intestines of conventionally raised mice, marked changes occurred in the germ-free mice within 2 weeks. Among other things, their body fat content increased and insulin resistance became apparent even though their food intake was reduced (Bäckhed et al., 2004). Evidently, more food was converted into fat and hence these mice gained weight. More than this, the microbiota was integral in the absorption of monosaccharides from the gut lumen (interior of the gastrointestinal tract), resulting in a process by which fatty acids are produced (lipogenesis) and then stored. Moreover, a type of protein "Fasting-induced adipocyte factor" (Fiaf) was suppressed in the intestinal epithelium, which is essential for triglycerides to be stored within adipocytes. In essence, these studies were among those that led the way in suggesting that the gut microbiota is fundamental in moderating energy being obtained from foods as well as subsequent energy storage.

MICROBIOTA AMONG SUPER-AGERS

The microbiome may contribute to both the physical deterioration that accompanies ageing, as well as to healthy ageing and extreme longevity. In older animals, it is not uncommon for gut dysbiosis to occur, leading to intestines becoming leaky, so that released bacterial products promote inflammation and immune dysfunction. Individuals with high circulating levels of proinflammatory cytokines, particularly TNF-α, are generally more frail and less independent, more vulnerable to some types of infection, and more likely to experience chronic illnesses. To be sure, it is possible that the TNF-α elevations initially came about because of the "wear and tear" associated with ageing, together with greater exposure to infections over the lifetime, which in turn, modified the microbiome, the presence of inflammatory factors, and neuronal processes leading to mental disability.

In older individuals, especially in response to stressors, gut permeability increases, accompanied by elevated circulating proinflammatory cytokine levels. As well, changes occurred in the levels of a particular microbial family Porphyromonadaceae, which has been linked to cognitive decline and affective disorders, and was associated with elevated anxiety in older mice (Scott et al., 2017). It seems that ageing may be accompanied by a shift of the microbial community toward a profile reminiscent of that apparent in inflammatory diseases, and may contribute to the development of behavioral and cognitive disturbances. In fact,

(*cont'd*)

in young rodents that received gut bacteria from old mice, chronic inflammation could be accompanied by elevated leakage of inflammatory bacterial factors into circulation (Fransen et al., 2017).

It is interesting that with normal ageing, certain bacterial species disappear and others become more common, which could contribute to healthy ageing. Specifically, dominant species are replaced with subordinate species and particular bacterial groups (e.g., Akkermansia, Bifidobacterium, and Christensenellaceae) are more prevalent or are enriched. Thus, among extremely healthy individuals who were 100 years or older, their microbiota constituency resembled that of healthy young people (Bian et al., 2017). Cognitive performance among healthy older adults was also linked to the presence of particular gut bacteria (Manderino et al., 2017). To be sure, these findings are simply correlational, but they nonetheless offer interesting hints regarding processes related to extreme longevity.

Among turquoise killifish, which have a relatively short lifespan (4–6 months), several genes located on sex chromosomes, were linked to longevity (Valenzano et al., 2015), which might speak to the greater longevity of females. It was particularly interesting that when older fish of this species consumed the poop of younger fish, they lived longer, raising the possibility that some bacterial factors present in young poop produced benefits for the older fish. In other studies using *Caenorhabditis elegans*, elimination of 29 bacterial genes increased longevity, and limited age-related diseases. These effects seemed to have been related to a substance, colonic acid, which affects the worm's mitochondria, thereby altering energy regulation (Han et al., 2017). These data raise the possibility that the link between bacteria and longevity is a causal one, at least in worms, but it's some distance from worms to humans.

Not long after these initial findings, it was demonstrated that in genetically obese (ob/ob) mice, microbial communities could be distinguished from those that appeared in lean animals (Ley et al., 2005). Most prominently, the firmicutes were increased by 50% and the bacteroidetes were diminished to a comparable extent among those who were obese. When microbes harvested from fat and lean mice were fed to germ-free mice, those that received microbes from obese donors exhibited a much greater increase of fat than did those who received microbes from the lean donors (Turnbaugh et al., 2006), pointing to the causal connection between microbial factors and fat storage. It was later demonstrated that predictable phenotypic changes were provoked by the transplantation of fecal microbiota from adult female human twin pairs discordant for obesity into germ-free mice that were maintained on low-fat chow. Specifically, body and fat mass, together with obesity-associated metabolic phenotypes, varied with the fecal bacteria cultures received (i.e., from the heavy or lean twin). Tellingly, when mice that had received an obese twin's microbiota (Ob) were housed with mice containing the lean co-twin's microbiota (Ln), the increased body mass and obesity-associated metabolic phenotypes in Ob mice was prevented, which was likely because of lateral transmission of microbiota (Ridaura et al., 2013).

Gut bacteria produce spores that can survive in open air, and can be transmitted from one person to another, causing dysbiosis in the

second individual (Browne et al., 2016). Ordinarily, the skin microbiome plays a fundamental role in protection from infection, allergies, and the provocation of inflammation, so that when skin microbiome dysbiosis exists, the impact of a parasite can be markedly elevated. Interestingly, in mice the disturbance of the skin microbial community can be transferred to cage-mates (Gimblet et al., 2017), and it is conceivable that people living within the same home, may share a similar microbiome, and thus may share illness vulnerabilities, as well as the propensity for weight gain.

Gut bacteria also exist that can favor weight loss, rather than weight gain. For instance, among lean mice, the bacteria Akkermansia muciniphila is far more common among lean mice than in mice that are prone to diabetes, and when these bacteria were fed to the obese mice they tended to lose weight and the warning signs of type 2 diabetes diminished (Plovier et al., 2017). In humans, prebiotics fed to overweight children, reduced the weight gains that would otherwise appear in growing children, which has important long-term implications given that childhood obesity is often carried into adulthood (Nicolucci et al., 2017). These findings, and others like them, suggest that the gut microbiome might provide a target for obesity treatments, and for the reduction of type 2 diabetes symptoms (Remely et al., 2016). At the same time, as we've already seen, gut bacteria comprise many different subtypes and the specific combinations that are present will dictate different phenotypes.

Factors That Affect Microbiota and Their Implications for Well-Being

The microbial community is negatively affected by poor life-styles, as we saw earlier in relation to food consumption and obesity. Especially harmful effects are elicited by antibiotics that kill useful bacteria along with those that are not our friends. It was estimated that one in five hospitalized patients experience adverse effects related to antibiotic treatments, including gastrointestinal, renal, or hematologic disturbances. Some common antibiotic treatments (e.g., amoxicillin and azithromycin) taken over a period of just 7 days can have pronounced and long lasting negative effects on gut microbiota diversity (Abeles et al., 2016), and when administered early in life, gut hypersensitivity may persist into adulthood (O'Mahony et al., 2014). Yet, there are instances in which some bacterial species may produce positive side actions. For instance, antibiotics could influence gut bacteria that might otherwise contribute to the development of brain disorders. It is likely that the individual's genetic background, along with exposures to environmental stresses, play a role in shaping the microbiome and hence, determining what long-term repercussions result from its disturbance.

It should be considered that antibiotic treatments can affect the transmission of microbial factors from a pregnant mom to her fetus (vertical transmission), and may thereby disturb protective qualities associated with bacteria (Bäckhed et al., 2015). Hence, offspring can bear the benefits or risks associated not only with the genes passed on to them, but also the microbiota they inherit.

The impression might be gained from Fig. 3.2 that each of the main contributing factors independently influences microbial factors and well-being. Ultimately, however, microbial functioning and intestinal immunity are shaped and maintained by multiple interactive processes. Each of the factors shown in the figure affects others, and additively or interactively, gut bacteria will be affected. It is also the case that commensal microflora can affect and interact with immune processes, which can influence nutrition, including the presence of short chain fatty acids and particular vitamins, which then feedback and affect the

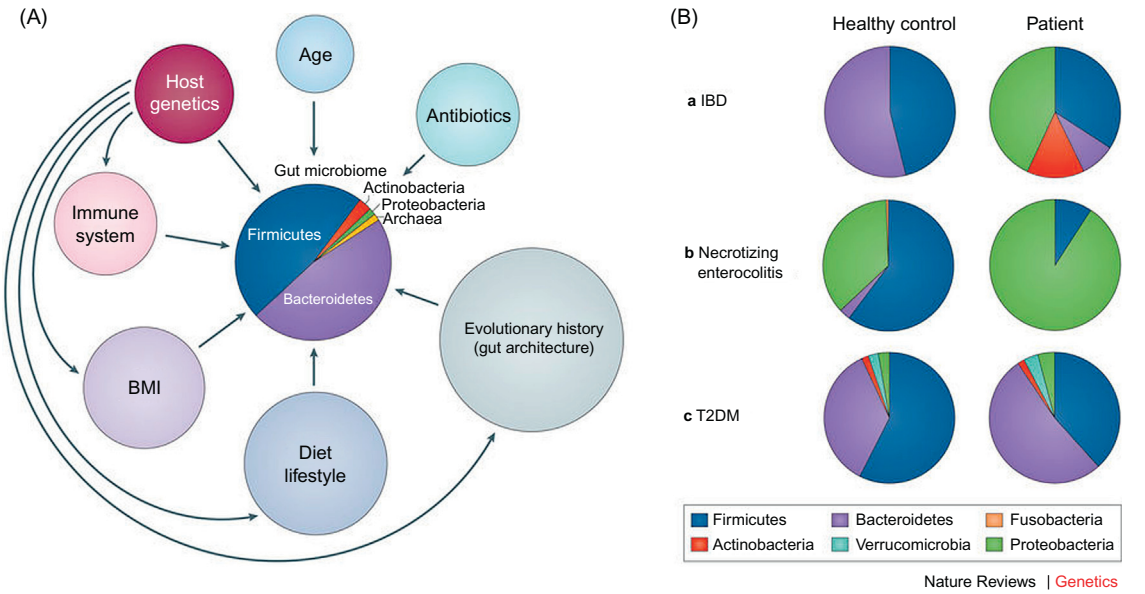

FIGURE 3.2 (A): The gut's physical architecture influences the microbiome constituency. Species that are phylogenetically related typically display more similar microbiomes than species that are only distantly related. Irrespective of age, diet, and geographic location, human gut microbiomes are more similar to one another (despite their own marked variability that stems mostly from diet and lifestyle factors) than they are to the gut microbiomes of other species. In general, the gut microbiome is readily affected by diet, age, genetic factors, host genetic factors, immune system functioning, and age, with the relative contribution of each of these being depicted by the size of their representation in the figure. The use of antibiotics also markedly influences the composition of the gut microbiome, essentially eliminating gut bacteria. The diversity of the gut bacteria in humans also varies with age. Prior to the age of 3 years, the gut microbiome is described as comprising limited species diversity, but takes on a more diverse, adult-like profile after the age of three. (B): Gut microbiome dysbiosis has been linked to many illnesses. As depicted in the figure, this has included inflammatory bowel disease (IBD) (part **a**), necrotizing enterocolitis (part **b**), as well disorders unrelated to the gut, such as type 2 diabetes (T2DM) (part **c**). In the case of each illness, the nature of the gut dysbiosis was very different. *Source: The left panel (A) and the Figure caption are taken from Hall, Tolonen, and Xavier (2017). The right panel (B) comes from Spor, Koren, & Ley (2011).*

microbiota (Spencer & Belkaid, 2012). By example, the presence of microbiota contributes to mast cell functioning following consumption of fat, which can then influence immune activity and instigate particular allergic and inflammatory responses (Sato et al., 2016).

Gut microbiota and the genes involved in regulating them (microbiome) are exquisitely sensitive to psychological stressors, and can be reversed by oral prebiotic treatment (Bharwani et al., 2017). Likewise, microbiota alterations can be provoked by prenatal stressors (Golubeva et al., 2015) and can markedly affect the development of immune processes. Conversely, intake of specific gut microbes can diminish the impact of stressors, such as strenuous exercise, and can limit abdominal dysfunction and discomfort associated with academic stressors. It also appears that in the absence of an inflammatory inhibitor NLRP12, the presence of beneficial bacteria were reduced and that of disruptive bacteria were elevated, leading to still more inflammation. As expected, increasing the presence of the good bacteria could terminate this negative cycle (Chen et al., 2017).

GETTING AROUND ANTIBIOTIC RESISTANCE TO C. DIFFICILE THROUGH FECAL TRANSPLANTS

The hospital-acquired antibiotic resistant bacterium C. difficile has proven to be particularly able to be transmitted from one individual to the next. If patients were treated with an antibiotic, even using the hospital bed that had recently been occupied by a C. difficile patient increased the risk of contracting this condition (Freedberg, Salmasian, Cohen, Abrams, & Larson, 2016). Not only was the occurrence of C. difficile infection more common among hospital patients treated with antibiotics, but variants of this bacteria emerged that were increasingly destructive owing to their ability to produce a toxin to destroy eosinophils in the gut that ordinarily act in a protective capacity (Cowardin, Buonomo, & Saleh, 2016). Making matters much worse, a new strain (NAP1) associated with multiple recurring C. difficile has been on the rise, doubling between 2001 and 2012.

The approach to deal with C. difficile comprised fecal microbiota being obtained from a healthy donor and then transplanted (in a purified form, most often by colonoscopy or through the nasogastric route, or more recently through acid-resistant capsules) to patients with resistant C. difficile. This results in reestablishment of a beneficial bacterial colony, and abatement of illness. The media has treated fecal transfer as a far-out procedure (probably because of the "yuck" factor), but it is hardly a novel one, as documented by de Groot, Frissen, de Clercq, and Nieuwdorp (2017) in a brief history of this topic. This approach was used as early as the 4th century in China to treat food poisoning and diarrhea, and was used elsewhere over the centuries to treat gastrointestinal problems. The 19th century Russian zoologist Metchnikoff proposed that balances of microbes within the colon, particularly elevated lactic acid bacteria, could lead to gut problems, and Nissle later extended this to include E. coli as a protective agent against Shigella and gastroenteritis. Somewhat later, fecal enemas were found to attenuate a form of colitis, and after a few years, fecal microbiota transplants were used in inflammatory bowel disease (Pigneur & Sokol, 2016). In animals, fecal transplants may also be effective in treating other resistant bacteria, including Eterococcus faecium and K. pneumoniae (Caballero et al., 2015).

Complete fecal transplants might not be necessary to deal with C. difficile, and potentially could be dealt with by transplantation of the bacteria C. scindens and three other bacteria (Buffie et al., 2015). Many factors present in feces, including colonocytes, archaea, viruses, fungi, and protists, may be fundamental to the effectiveness of the treatment and could potentially be enhanced by particular probiotics (Spinler, Ross, & Savidge, 2016). Should the mix of bacterial and nonbacterial components that lead to positive effects be identified, then it will be possible to generate treatments more efficiently and without having to rely on poo donors.

Psychological Functioning Associated with Microbial Changes

The potential involvement of gut microbiota in relation to multiple disease states instigated a great number of studies that traced the links between the microbiome, immune functioning, and brain neuronal changes, which might contribute to psychiatric disorders (Kennedy, Cryan, Dinan, & Clarke, 2017). Some psychological disturbances, such as mood disorders, may appear owing to variations of serotonin

formed in the digestive tract, or neurotoxicity brought about owing to increased metabolites of bacterial enzymes, such as D-lactic acid and ammonia (Galland, 2014). There is also ample evidence pointing to the microbiota having an impact on depressive-like features (see Chapter 8, Depressive Disorders). For instance, as adults, mice that had been born germ-free, exhibited altered dendritic morphology in the amygdala and hippocampus that were linked to depressive-like features (Luczynski et al., 2016). Likewise, among nonobese diabetic mice, gut microbiota could drive depressive-like symptoms (e.g., social avoidance), which could be attenuated by antibiotic treatment, and then resurrected through reconstitution of the microbiota from donor mice (Gacias et al., 2016).

Multiple routes have been identified (as shown in Fig. 3.2) by which microbiota affect the brain and thus could promote psychopathology. Being a major pathway between the gut and brain, the vagal nerve was implicated as a player in accounting for anxiety and depressive-like behaviors, possibly acting through anti-inflammatory processes (Borovikova et al., 2000). As we'll see in later chapters, various hormones, neurotransmitters, growth factors, and immune related molecules that have been linked to depression are influenced by the microbiome. Moreover, ss described in Chapter 1, Multiple Pathways Linked to Mental Health and Illness, enterochromaffin cells of the gut epithelium can release serotonin and can activate CNS functioning (Bellono et al., 2017). In addition, peripheral cytokines are altered by gut bacteria, and manipulations of the gut microbiota can affect serotonin levels and specific serotonin receptors, norepinephrine, dopamine, and GABA activity in limbic brain regions, thereby influencing mood states (e.g., Clarke et al., 2013; Stilling et al., 2015). In addition, gut bacteria can influence neuroendocrine factors (e.g., CRH) and neurotrophins (e.g., BDNF) within brain regions that are sensitive to stressors and which have also been tied to the development and maintenance of depressive disorders. Further to this, strong immunogenic agents engender prounced corticoid responses, which can diminish the effectiveness of the gut barrier (Söderholm & Perdue, 2001). The migration of bacteria from the gut will thereby be facilitated, and these bacteria may promote the production of immune signaling molecules (cytokines), that instigate mood disturbances (Maes, Kubera, & Leunis, 2008).

In Chapter 8, Depressive Disorders, where we'll consider the processes associated with depressive illness, it will become clear that a balance exists amongst commensal bacteria so that some are aligned with the development of illness, whereas others seem to act in a protective capacity by acting against inflammatory processes. In line with this, among rats highly vulnerable to depressive-like states, probiotic treatment may counter behavioral disturbances that may have been provoked by proinflammatory changes instigated by a high fat diet (Abildgaard et al., 2017). As we'll see, as well, antibiotics can affect mood states by altering microbiota, but also affect processes beyond the microbes that they target. This includes their well documented effects on mitochondrial functioning, microglia reactivity, and factors important for neuroplasticity, such as mTOR, which collectively can impact many processes aligned with mental illness. Although antibiotics have been a primary concern in relation to microbiota changes and dysbiosis, such treatments are hardly alone in affecting microbiota within the gut as well as elsewhere. Indeed, nonantibiotic drugs, such as antipsychotics, antidepressants, nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, statins, metformin, and proton pump inhibitors (PPIs) were found to influence a wide range of microbiota classes. The secondary effects of some of these have been suggested to influence the frequent weight gain and visceral fat provoked by antipsychotioc mediction (Le Bastard et al., 2018).

Microbiota and Immunity

The gut is a dirty place, being the recipient of various foods, some of which may be contaminated, and a good portion of our immune cells operate within the gut. Intestinal immunity comprises collaboration between specific types of immune cells and many different cytokines, various nutritional factors (e.g., short chain fatty acids, particular vitamins), and commensal bacteria. Moreover, reciprocal communication occurs between gut microbiota and CNS processes, and consequently gut-level disturbances can instigate psychological, metabolic, and immune-related disorders (O'Mahony, Clarke, Dinan, & Cryan, 2017a).

Microbial factors are fundamentally involved in the development of immunosuppressive responses generated by regulatory T-cells (Treg) to dietary antigens (Kim et al., 2016). This, of course, is critical for the prevention of excessive immune responses being generated in the face of normal dietary intake. It is thought that gut dysbiosis may promote disturbances of Treg cells and imbalances of Th1, Th2 and Th17 lymphocytes, which can promote autoimmunity to antigens derived from the diet and may contribute to autoimmune diseases. Even the regulation of neuron myelination within the prefrontal cortex (Hoban et al., 2016) is affected by the microbiome, and thus might dispose individuals to susceptibility to multiple sclerosis (Mangalam et al., 2017).

Beyond the effects on neuronal processes, the microbiota can affect brain microglia, and through the release of cytokines can affect psychopathological conditions. Furthermore, pre- and probiotic manipulations are capable of altering glycemic dysregulation, such as glucose tolerance and insulin resistance, which was accompanied by elevated plasma levels of the anti-inflammatory cytokine IL-10. It was thus suggested that microbial manipulations could potentially influence any illness that involves ongoing inflammatory or metabolic disturbances (de Cossío et al., 2017)[4].

An interesting meta-analysis that considered ten diseases indicated that the number of microbiota genera varied with different illnesses. Whereas some illnesses were associated with the presence of numerous genera, others appeared to be associated with a lack of particular microbiota, and many microbiota were linked to multiple illnesses. In effect, some of these conditions are not disease-specific and are general vulnerability factors, so that other elements contribute to the specific illness that emerges. Such findings might be a step in providing information that could be relevant to the use of probiotics and prebiotics (whereas probiotics comprise living micro-organisms, prebiotics are food ingredients that induce the growth or activity of beneficial microorganisms) in relation to specific illnesses.

As much as the findings concerning germ-free mice are interesting, their relevance for neuroimmune processes are, in some ways, not as clear as one might like, especially as the blood–brain barrier (BBB) may be disturbed in these mice (Braniste et al., 2014). Thus, endogenous circulating immune cells and any microbial species that might escape the confines of the gut could potentially access the brain. As well, this would result in exceptional vulnerability to a range of potentially toxic insults that are present in the periphery following environmental exposure. Remarkably, exposing germ-free mice to the normal microbial constituents of the gut obtained from normally housed

[4] As we'll emphasize repeatedly, caution should always be exercised in embracing any emerging therapies. This is particularly evident with regards to "hot" areas of research, where there might be the inclination to "jump on the bandwagon." Microbiota have been implicated in a very large number of illnesses, and there may be the concern, as often stated, that when something explains everything, it explains nothing.

mice, reversed BBB deficits. In fact, even treating them with the short chain fatty acid metabolites from normal microbiota appeared to repair the BBB deficits, such that tight junction integrity was increased, which was also associated with the prevention of protein (e.g., IgG) infiltration into the brain parenchyma.

The fact that constituents of the microbiota send signals to the brain that affect BBB functioning and brain homeostasis, has wide ranging implications for virtually all neuronal pathologies. This is illustrated by the finding that the microbiota influences the development of infection following stroke (Stanley, Moore, & Wong, 2018). In fact, when fecal samples from a mouse that experienced ischemic stroke were transferred to other mice, the size of a stroke-induced neuronal infarct was increased in the microbiota receipients (Singh et al., 2016). Conversely, antibiotic treatment reduced stroke damage and diminished the infiltration of inflammatory T cells (Benakis et al., 2016). These findings suggest bi-directional communication between the microbiota and brain, and that the translocation of microbial elements might infiltrate the brain or other organs to influence their functioning.

It is highly likely that the complex interactions between the differing microbial species might contribute to autoimmune disorders. Germ-free mice with impaired BBB integrity were found to be more vulnerable to the development of autoimmune pathology in an experimental autoimmune encephalitis (EAE) model of MS. Furthermore, having the normal gut commensal bacteria was essential for mounting CD4 + T cell and B cell antibody responses in a model of relapse remitting MS (Berer et al., 2011), and thus germ-free mice might be at greater MS vulnerability. In parallel with such germ-free studies, antibiotic-induced microbiota changes also impacted MS pathology. In MS patients marked gut dysbioisis was present, particularly reductions of clostridium and Bacteroidetes species (Miyake et al., 2015). A further interesting aspect related to autoimmune disorder is that fusobacteria increased relapse rate (Tremlett et al., 2016), but successful treatment of MS was associated with elevations of Prevotella and Sutterella species (Jangi et al., 2016). Thus, various microbial species likely have differing effects on brain processes and that their collective impact depends upon a delicate balance between them and the metabolites they excrete.

Physical Illness, Immunity, and Gut Bacteria

The link between microbiota, immune functioning, and disease conditions has been supported by the finding that germ-free mice raised in a sterile environment lived longer following a skin graft, possibly because immune functioning was diminished and hence foreign tissue was not attacked. Conversely, if these mice received microbes from untreated mice, they rejected the skin graft more readily. In essence, these data point to the importance of the microbiota in determining immune functioning and tissue rejection (Alhabbab et al., 2015). Paralleling these findings, tissue transplants involving lungs, skin, and intestines, which had been exposed to external influences, were less successful than transplants of tissues that were less directly affected by external microbial factors (Lei et al., 2016).

Variations in immune function are likely pertinent in the links between the microbiome and development of physical illness such as chronic kidney disease (Nallu, Sharma, Ramezani, Muralidharan, & Raj, 2017). The gastrointestinal tract shares reciprocal connections with the immune system. In Chapter 2, The Immune System: An Overview, we saw that immune cells have membrane pattern recognition receptors (PRRs) that mediate recognition of damage and pathogen-associated molecules (PAMPs), and damage-associated molecular patterns

(DAMPs). These PRRs are rudimentary aspects of the immune system that evolved, in part, as a way of detecting pathogens or other microbial threats, and may operate to enable microbiota to communicate with the immune system (Chu & Mazmanian, 2013).

Generally, PAMPs are initial sensors that allow immune cells to recognize microbial presence, and determine their pathogenic valence (e.g., commensal microbes can often be tolerated). The toll like receptors (TLRs) and NODs (nucleotide-binding oligomerization domain-like receptors) are among the most prominent PAMPs and are found throughout the brain and immune system. They have evolved to recognize specific motifs that characterize bacterial, viral, or fungal invaders. Upon their recognition, very robust intracellular pathways are engaged that give rise to the mobilization of defensive inflammatory (e.g., cytokines), enzymatic, and oxidative (e.g., superoxide) factors, depending upon the nature of the threat. DAMPs act in a similar fashion but in the absence of a microbial constituent, instead becoming active following the detection of specific factors that are released in response to cellular distress. Among these distress signals, adenosine triphosphate (ATP), and other purines, along with mitochondrial and other intracellular factors are released into the extracellular space in the presence of a damaged cell, creating a "sterile" inflammatory reaction.

A distinction has been made between PAMPs and the largely interchangeable term, microbe-associated molecular patterns (MAMPs), which respond to various microorganisms and also act as a bridge between the enteric nervous system and innate immune system. The MAMPs, by virtue of their effect on immune cells, modulate inflammation (Chu & Mazmanian, 2013) and might even influence microbial interactions with TLRs (Zhou et al., 2015). Some of these interactions may not always lead to pathological or inflammatory conditions, which would be in keeping with the symbiotic relationship between the gut microbiota and the host organism.

However, problems may arise when microbial dysfunctions result in improper "sensing" of microbiome signals. If such protective processes are not doing the job effectively, excessive immune activation and chronic inflammation may evolve (Chu & Mazmanian, 2016; Chu et al., 2016). Fortuntely, we are blessed with a gene (SIGIRR) that operates to stimulate immune responses that interfere with bacteria forming colonies that would ordinarily have negative health effects. Disruptions of SIGIRR owing to antibiotic treatments, can cause dysbiosis wherein the battle for supremacy moves toward the side of the harmful bacteria (Sham et al., 2013).

As we've seen, microbial dysbiosis has been implicated in a number of diseases that involve gastrointestinal processes and eating disorders, such as anorexia nervosa and bulimia (Chu et al., 2016). Beyond these conditions, gut bacteria have also been linked to metabolic dysfunctions (e.g., insulin resistance), ultimately promoting the development of type 2 diabetes, and it has been proposed that the disturbed balance of gut bacteria might serve as a target in the treatment of this illness. Altered bacterial levels have also been associated with increased proinflammatory activity (e.g., IL-17) that exacerbates autoimmune conditions (López, Rodríguez-Carrio, Caminal-Montero, Mozo, & Suárez, 2016). Still other illnesses might come about because the wound-healing capacity associated with the microbiome might not be operating properly or might not be present. A wide range of other illnesses, which will be discussed in ensuing chapters, have also been linked to immune disturbances that might have their roots in microbial dysbiosis and inflammatory processes. These comprise cardiovascular illnesses, periodontal disease, rheumatoid arthritis, and allergies, as well as seemingly unrelated illness conditions, such as

chronic kidney disease, uremic toxicity, multiorgan failure, and several forms of cancer (e.g., Chen et al., 2016). The microbiota has also been associated with the accumulation of amyloid proteins that were linked to neurodegenerative disorders (Chen et al., 2016), which may also be linked to inflammatory processes (see Chapter 14: Inflammatory Roads to Parkinson's Disease and Chapter 15: A Neuroinflammatory View of Alzheimer's Disease).

SICKNESS FEATURES ASSOCIATED WITH SYSTEMIC INFLAMMATION STEMS FROM BRAIN CHANGES

Sickness behaviors in rodents (diminished social interaction, ruffled fur, hunched posture, inactivity, sleepiness) are typically associated with the administration of LPS or cytokines, such as IL-1β, and have been taken to model some of the symptoms associated with depressive disorders. Characteristics of sickness behaviors are frequently apparent among patients experiencing autoimmune disorders, likely reflecting elevated inflammatory immune activation. It seems that in the context of organ inflammation, increased TNF-α levels give rise to monocytes being recruited to the brain, thereby increasing microglial activation and the production of sickness behaviors. The sickness profile associated with liver inflammation in mice can be diminished by a probiotic treatment without affecting severity of the illness, the actual gut microbiota composition, or permeability of the gut, but were tied to diminished microglial activation, and cerebral monocyte infiltration (D'Mello et al., 2015). The sickness behavior and its resolution by probiotics may thus involve brain processes, leading to the possibility that altering systemic immune functioning or microglial activation, as well as limiting recruitment of monocyte-secreting TNF-α within the brain, may diminish some of the features of systemic inflammatory diseases.

Caveats Concerning the Potential for Using Microbiota for Health Benefits

With the increased understanding regarding the contribution of microbiota to illness occurrence, one might be seduced into thinking that we may be on the cusp of being able to target microbiota in order to diminish or prevent illness. Although there have been reports consistent with this perspective (as in the case of rheumatoid arthritis) (e.g., Marietta et al., 2016), in the main, the positive effects observed were modest, and altering microbiota through diets or by probiotics, did not have sufficiently powerful effects to moderate systemic inflammation. Furthermore, it has proven difficult to identify specific bacteria that cause the appearance of illnesses, mainly because so many processes are linked to different pathological conditions. Very many bacterial species exist, each with thousands of genes, and they may interact with numerous hormonal and gut-related processes, and can be modified by multiple environmental and experiential influences. The treatment and prevention of illnesses may also be subject to dynamic processes that are affected by multiple environmental influences, and these vary appreciably across individuals. Accordingly, potential treatments will no doubt have to comprise multiple bacterial changes, rather than any one or two bacteria, and the contribution of treatments may well vary over time (Lynch & Pedersen, 2016).

Even when the processes leading to a disease have been identified, it shouldn't be expected that manipulating these processes would necessarily attenuate the chracteristics of the condition. Once an illness is sufficiently advanced, or particular factors well entrenched, simply

altering the microbiome can help in limiting further illness progression, but might be insufficient to reverse the already existing damage. It should be added that aside from the other actions that have been ascribed to microbiota, they are also involved in the production of metabolites that enter into circulation, which then affect various conditions outside of those involving the gut itself. For the moment, firm conclusion regarding the effectiveness of probiotics in the treatment of most illnesses ought to be held in abeyance (Bravo-Blas, Wessel, & Milling, 2016).

These caveats notwithstanding, there are excellent possibilities of being able to capitalize on individual differences in relation to health risks. It has been maintained that individuals can be stratified based on a few dominant bacteria, and thus the broad variability often discussed in relation to microbiota may be somewhat more limited. It may be possible to use these broad classes of bacteria in designing ways (e.g., through prebiotics, probiotics, or synbiotics – the latter contains both pre-and probiotics) to enhance gut bacterial functioning (Cani & Everard, 2016). People who consume the same diet, may nevertheless present with glycemic responses that differ appreciably, possibly owing to individual differences in gut microbial composition. Thus, finding appropriate diets for any given individual might benefit from a personalized approach, which could include glycemic responses, microbial factors, and genetic contributions. (Zeevi et al., 2015). For instance, among young women, socioeconomic factors, specific food choices, such as fat intake, and the presence of a gene variant (DRD4 VNTR), together could predict susceptibility to obesity (Silveira, Gaudreau, Atkinson, Fleming, & Sokolowski, 2016). Obviously, using a personalized approach to deal with diets and obesity would be enormously difficult (and financially constraining), but given the obesity crisis that seems to be escalating, such an approach could have both short- and long-term benefits.

Moderating Variables Concerning Gut Bacteria and Health Outcomes

It cannot be emphasized enough that any positive (or negative) influences of microbial factors may be dependent on a constellation of genetic, experiential, and psychosocial factors (life experiences, trauma, and social learning). The cumulative effects of these varied factors can also affect the effectiveness of intervention and treatment strategies. Just as stressful events can affect microbiota and/or immune functioning, and hence the provocation of disease, exposure to bacteria can affect the subsequent response to a stressor, thereby affecting physical ailments and psychological disturbances.

Microbiota variations are influenced by the presence of particular inherited genes (Goodrich et al., 2016), foods eaten, and epithelial cells that line the various cavities and surfaces of multiple structures. As already indicated, there is a constant battle within the gut between bacteria that try to cooperate and those are antagonistic with one another. Foods eaten fuel these processes so that when the needs of microbes are consistent with the needs of the host, well-being ensues; however, when these needs are at odds with one another (as occurs in response to sugars and fats), the antagonistic relationship may lead to illnesses. In the latter instance, microbes may begin to use nutrients that the body requires (e.g., iron), resulting in the immune system activity increasing to deal with these microbes, potentially leading to inflammation, obesity, and diabetes. In addition, the use of antibiotics have had an enormous impact on disturbing microbial diversity and hence affecting resilience and vulnerability to illness (Belkaid & Hand, 2014).

Beyond these many linkages, gut bacteria may also influence neurogenesis. Specifically, eliminating gut bacteria through antibiotics can diminish hippocampal formation of new neurons and disrupt performance in memory tasks. Interestingly, mice that had exhibited

memory disturbances displayed lower white blood cells, primarily monocytes that carried Ly6Chi as a marker, implicating a link between gut bacteria, aspects of immune functioning, and brain neurogenesis. This connection was confirmed by showing that these outcomes could be reversed by reconstitution with normal gut flora provided that mice were also able to exercise (using a running wheel) or given probiotic treatments (Baruch & Schwartz, 2016). In addition to affecting hippocampal neurogenesis, disrupting the microbiome in mice though antibiotics also affects hippocampal glial reorganization, thereby favoring the development of depressive-like features (Guida et al., 2017).

CONCLUDING COMMENTS

Bacteria and viruses have been constant concerns, but in several ways they have become more threatening. Many vaccines aren't as effective as they ought to be (e.g., in the case of influenza vaccines) and the evolution of antibiotic resistant bacteria have become more apparent in relation to a number of existing diseases. Of course, the possibility of new emerging diseases seems to be more a certainty than a possibility. Beside the obvious consequences of infection, it has become apparent that activation of inflammatory processes may promote multiple diseases, including physical and psychological disorders.

Although it had been suspected for well more than a century, it has only recently been established that bacteria and other microorganisms exist throughought the human body, serving to maintain well-being. When dysbiosis occurs, vulnerability to multiple illnesses may occur, and some of the agents, such as antibiotics, which protected us from bacterial infections, may have acted against us by disruption of the gut microbiota. The increased knowledge regarding microbiota has also provided us with tools that could be used to enhance health. Specifically, it is now understood that the human gut is not equipped to digest the many macronutrients that are consumed, such as plant polysaccharides. Thus, commensal bacteria, such as Lactobacillus and Bifidobacterium, are involved in doing the job, ultimately producing short chain fatty acids (SCFAs), such as butyrate, lactate, and propionate. These factors may affect immunity, possibly diminishing potentially pathogenic microbes and augmenting gut barrier function (Slavin, 2013), and they may have neuroprotective effects (Horn & Klein, 2013). The actions of short chain fatty acids on CNS processes are only beginning to be understood, but it seems that some fatty acids (e.g., sodium butyrate) can attenuate stressor-provoked serotonin and BDNF alterations (Sun et al., 2016) and may thereby influence the function of microglia (Erny et al., 2015) and hence affect psychological functioning.

In view of the health benefits (and risks) associated with gut bacteria, there has been an obvious effort to enlist the microbiome to enhance well-being. In this regard, the lifestyles that are often adopted could produce microbial dysbiosis thereby promoting psychological disturbances, which can be attenuated, at least to some extent, by pre- and probiotic consumption. The prebiotics that have been used to modify gut-related disorders, may have their effects owing to multiple changes that evolve. These include the production of antimicrobial compounds, growth substrates, such as vitamins and polysaccharides released into the internal environment, reduction of the luminal pH, prevention of particular microbes from adhering to epithelial cells, augmented barrier functioning, and modulation of immune responses (Power, O'Toole, Stanton, Ross, & Fitzgerald, 2014). Prebiotics, such as certain oligosaccharides in human milk can also inhibit monocytes, lymphocytes, and neutrophils from binding to endothelial cells, and might thereby contribute to the relatively low

frequency of inflammatory diseases in milk-fed human infants (Bode et al., 2004).

The microbiota present, as we've seen, varies greatly across individuals, being affected by many environmental factors, including diet, stressors, and environmental toxicants. But, the individual variability that exists rearding microbiota, coupled with the many factors that affect microbiota balances, makes it difficult to discern what reflects a harmful versus a beneficial compliment of bacteria. It has indeed been suggested that in relation to treatment of illness, the intestinal microbial population might need to be individually tailored by diet and other manipulations (Shoaie et al., 2015). But, it's still uncertain which good bacteria to call upon, how much of it is needed, and how to do battle with bad bacteria. While the actions of some bacteria are known, many others are hardly understood, but their positive (or negative) actions are being uncovered.