Chapter 16
Evolutionary Principles and Host Defense

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Lay Summary On multiple timescales, evolution plays critical roles in the processes by which humans respond to infectious agents and other foreign substances and the ways that infectious agents try to evade immune mechanisms and exploit the resources of the hosts they infect. The human immune response depends on white blood cells known as lymphocytes, and the success of the responses these cells mount against such well-known causes of infection as HIV-1 depend critically on cellular proliferation, variation in antigen receptor genes, and selection, i.e., differential survival and replication of cells that differ with respect to these immunity-related genes. Similarly, the capacities of bacteria, viruses, and other infectious agents to harm hosts in transforming host resources into pathogen progeny, to transmit to new hosts, and to evade drugs and vaccine responses depend critically on mutation and selection, i.e., pathogen evolution. Some of the evolutionary changes that affect the abilities of infectious agents to successfully infect hosts, replicate, and transmit to additional hosts can occur within the time frame of a single host infection, creating a sort of 'arms race' between the pathogen and the cells of the host immune system.

16.1 Introduction: Evolutionary Timescales and the Immune System

Practitioners of the medically important fields of immunology and microbiology have recognized the relevance of evolution for their disciplines for over a century [1, 2]. As understanding in these fields has progressed to characterizing the roles of cells, genes,

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and molecules in determining the specificity, magnitude, and quality of immune responses, and the molecular mechanisms responsible for pathogen-mediated tropisms, virulence, transmissibility, and resistance to pharmacologic agents, the relevance of evolution has become even more sharply defined. In this chapter, the goal will be to employ selected examples to illustrate some of the many aspects of host–pathogen relationships for which knowledge of evolutionary mechanisms is relevant and arguably essential.

Evolution shapes the human immune system and the immune response on three timescales (Fig. 16.1). First, over millennia, phylogenetic evolution produced extraordinarily complex and highly networked sets of cellular and molecular responses in vertebrates, to both external and internal stimuli, that we generally refer to as the immune system [3, 4]. This system includes responses that depend on receptors, known as innate immune receptors, for microbial components that are present, with relatively modest structural variations, in many species of pathogen [5, 6]. These structures are often referred to as pathogen-associated or (more accurately) microbial-associated molecular patterns (PAMPs or MAMPs). The innate immune receptors for these microbial molecules are typically referred to as pattern recognition receptors (PRR).

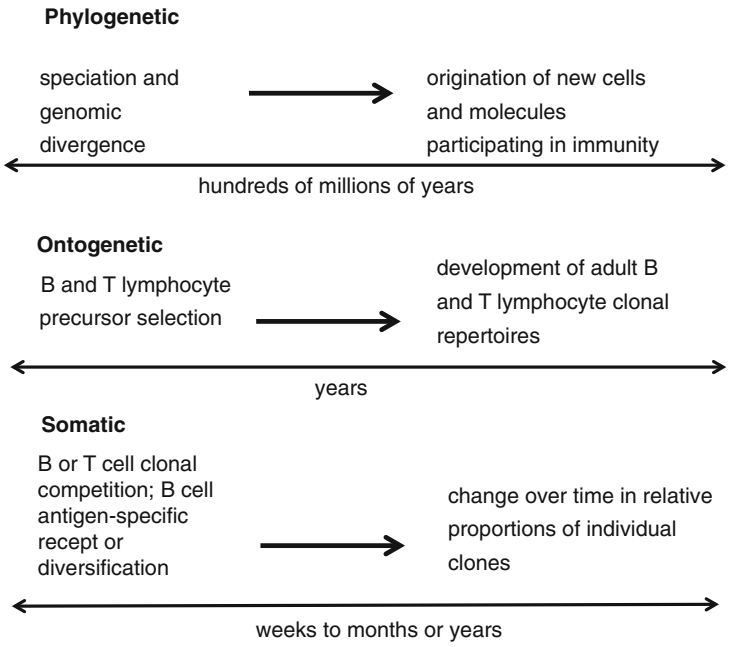

Fig. 16.1 The immune system evolves in clinically relevant ways on three timescales: phylogenetic, ontogenetic, and somatic

Second, the antigen-specific cells of the immune system, B and Tlymphocytes originate primarily from hematopoietic stem cells in the bone marrow, like other cell types found in the blood. They develop into mature forms in primary lymphoid organs (bone marrow for Bcells and thymus for Tcells) through a complex process of diversification of the genes encoding antigen-specific receptors (ASR) and selection. Differential fitness arises in part from differences in antigen-specific receptor (ASR) amino acid sequence and, therefore, antigen specificity [7, 8]. Over a period of weeks to months, these ontogenetic selection events, both positive and negative, determine which B or Tcells contribute to the naïve immune repertoire, which corresponds to a sort of dynamic library of receptor structures that confer on the organism its unique abilities to respond to exogenous molecular stimuli in the form of pathogens and their components, toxins, and other substances originating from non-human species. These processes represent evolution of populations of somatic cells, not populations of organisms. They are relevant to medicine both because they establish the ASR repertoires for B and Tlymphocytes and because aberrations of these developmental pathways can lead to deficiency or malignant diseases.

Third, immune responses involve competition among white blood cells, either B or Tlymphocytes, expressing ASR differing in amino acid sequence. Typically, at a given time, two or more B or Tcells may share the same precise ASR amino acid sequence. Cells displaying ASR with structurally identical antigen-recognition domains are referred to as a clone. Thus, the immune response can be viewed as a process in which B or Tcells displaying ASR with different amino acid sequences (encoded by genes with different nucleotide sequences) exhibit differences in fitness, i.e., a process of clonal competition [9–12]. Particularly in the case of Blymphocytes, the cells that produce antibodies, the clonal competition underlying an immune response can involve both ongoing mutation (through a unique process known as somatic hypermutation) and selection, and represents a truly neo-Darwinian evolutionary process played out (i.e., on the level of somatic cells as opposed to independent organisms), like the processes critical for B and Tlymphocyte repertoire development. This physiological form of evolution occurs in a time frame of days to weeks or months as opposed to the years, centuries, or millennia generally associated with phylogenetic evolution.

The outcome of this somatic cell competition can make the difference between life and death in the setting of an infection by a pathogen for which neutralizing or opsonizing antibodies provide the main mechanism of protective immunity and that produces progeny at a rate comparable to or more typically much greater than the rate at which Blymphocytes proliferate. In theory at least, a tenfold increase in average antibody affinity from an initially modest value can substantially reduce the time necessary to reach the threshold of protective antigen-binding activity by a time interval in which the pathogen burden could increase substantially. Blymphocytes, in mice, require on the order of 7 h to divide [13] while bacterial pathogens can divide as frequently as every half hour and viruses can increase at even more impressive exponential rates. Thus, the selection of somatic cells can influence the fitness of the whole organism and the trajectory of organismal evolution.

16.2 Research Findings and Implications for Policy and Practice

16.2.1 B- and T-Cell Evolution and HIV Vaccine Development

A particularly salient example of the medical relevance of B-cell and immunoglobulin gene evolution is in the context of HIV vaccine development. HIV-1 has an exceptionally high mutation rate [14] and a proclivity for recombination [15]. These two attributes contribute to extremely rapid genomic diversification [14] and evolution, both within-host and between-host [16]. The scale of HIV-1 genomic diversity is exemplified by the claim from Korber et al. that the HIV-1 viral genomes in one infected individual encompass the same approximate extent of nucleotide sequence diversity exhibited by the worldwide population of influenza A viral genomes over the course of a year.

Although a single virus is responsible for the transmission of HIV in most cases [17], diversification of the virus in individual hosts implies that different subjects are likely to be infected by genetically distinguishable viruses. Therefore, a vaccine that elicits antibodies that interact effectively with only a subset of circulating HIV viruses is unlikely to be highly effective on a population basis. These realities have prompted intense interest in identifying and characterizing what are known as potent broadly neutralizing antibodies (pbnAb) that will prevent infection of host cells by the vast majority of extant HIV viruses. A number of prominent investigators are hoping to be able to design HIV-derived immunogens (i.e., viral proteins that can stimulate an immune response) that can elicit pbnAbs.

What has been revealed in studying numerous monoclonal human pbnAb is that they have a very high number of somatic mutations (see Glossary) in the variable domains [18–20], which are the portions of antibodies that are primarily responsible for determining the affinity and specificity of interactions with antigens. Somatic mutations only in the portions of immunoglobulin genes that determine the amino acid sequences of variable domains result from a process that couples highly localized (i.e., affecting only variable and not other antibody domains) genetic variation with intense clonal competition and selection based on access to follicular helper T-cells. These helper cells are CD4+ Tlymphocytes that provide signals critical to activating the somatic hypermutation mechanism (see Glossary) and also isotype switching. These processes are most often, although not always, localized to specialized structures in secondary lymphoid tissues known as germinal centers (GC) [21]. Consequently, the antigen elicits an intense evolutionary process that offers one of the several currently plausible pathways to developing a vaccine that can counter the rapid evolution of HIV-1 [22] (Fig. 16.2).

In the case of HIV-1, only a minority of infected individuals develops pbnAbs, and in these individuals, it can take two or more years for pbnAbs to be produced. At that late stage of infection, these antibodies cannot eliminate the virus from all infected cells, some of which harbor the virus in latent forms that do not express

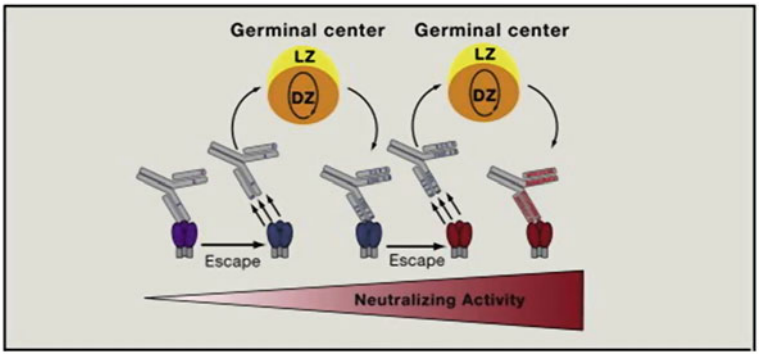

Fig. 16.2 Coevolutionary conflict between HIV and host Blymphocytes and antibodies. Selection of viral envelope variants (indicated by *trimer color* change) by neutralizing antibodies leads to altered viral envelope proteins that in turn select for altered antibody V domains. Selected amino acid substitutions confer better binding along with more potent and broader neutralizing activity. These events occur in the germinal centers (GC) of lymph nodes or other secondary lymphoid tissues. During this process, the Blymphocytes involved proliferate in the *dark zone* of the GC and interact with helper T-cells in the *light zone* (LZ) of the GC. Reprinted from [46] with the permission from RightsLink

sufficient quantities of viral protein for the antibodies or other elements of the immune response to recognize and destroy these cells. In contrast, in the vaccine context, pbnAbs would be present in the circulation prior to initial infection, thereby permitting the antibodies the chance to prevent infection at the start.

Of course, similar if less extensive evolution of Bcells and immunoglobulin genes are associated with antibody responses to most protein antigens. Such evolution is undoubtedly of importance for protective antibody responses elicited by vaccines for many bacterial and viral pathogens.

T-lymphocytes do not exhibit somatic hypermutation (or class switch recombination) but they still engage in intense clonal competition. Therefore, cell-mediated immune responses (which are mediated by T-lymphocytes) can also display changes over time in relative proportions of different clonal lineages; i.e., T-cell populations also evolve in a neo-Darwinian sense in the time frame of an immune response. A particularly interesting example of this process leading to an autoimmune disease, scleroderma, in patients with cancer was described recently [23]. Mutations in a gene that encodes a subunit of RNA polymerase III (an enzyme involved in synthesizing ribosomal and transfer RNA molecules) appear to facilitate tumorigenesis and also contribute to the elicitation of CD4+ T-cell responses that cause the pathology associated with scleroderma. Both the process of tumor formation and the immune response to the new antigen generated by the tumor-promoting mutations in the cancer cells represent examples of somatic cell evolution with clinical consequences.

16.2.2 Pathogen Evolution

The evolution of pathogens affects many medically relevant attributes including pathogen virulence, transmissibility, drug resistance, and the effectiveness of vaccines (Fig. 16.3). Below, I briefly provide examples for each of the above.

16.2.2.1 Virulence

Consider *Toxoplasma gondii*, which is an intracellular protozoan parasite that infects many different vertebrate species asexually and undergoes a sexual cycle after infecting cats [24]. Parasite oocysts are potentially introduced into the human environment in cat feces. *T. gondii* is of interest in clinical medicine because humans can serve as accidental intermediate hosts when they ingest oocysts in, for example, undercooked, contaminated meat or ingest mature parasites in contaminated drinking water. Mother-to-child transmission can also occur.

In most healthy individuals, the infection does not cause illness, but in individuals with immune deficiencies and in fetuses, it can cause substantial morbidity. In the case of congenital infection of a fetus, morbidity, including vision loss, cognitive deficits, and seizures, tends to be more severe with earlier infection. Fetal infection can result in either miscarriage or stillborn birth. Sibley and colleagues [25] have now further clarified the molecular basis for the variation in virulence among different *T. gondii* lineages for mice, an important prey species for cats and therefore an important intermediate host species.

There are three lineages of *T. gondii* in North America and Europe, and these lineages vary substantially in virulence for mice. With respect to typical laboratory mice, Type I is highly virulent, Type II exhibits an intermediate degree of virulence, and Type III is avirulent. In previous work [26, 27], Sibley and his associates used

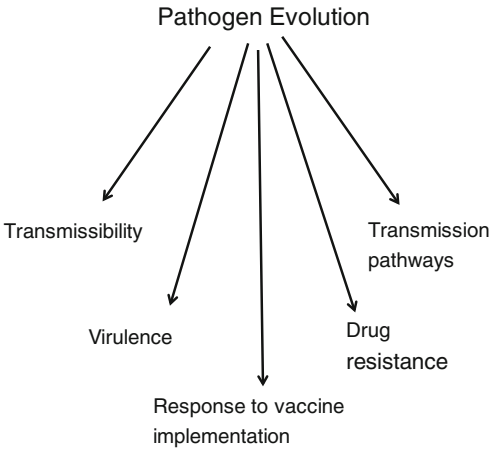

Fig. 16.3 Pathogen evolution influences clinically relevant traits such as transmissibility, virulence, drug resistance, and adaptation in response to the introduction of vaccines. Insights into pathogen evolution can provide a means of determining the origins and pathways of spread for infectious disease outbreaks

genetic crosses among these three lineages to identify key genes that contribute to virulence. Specifically, they identified a gene (*ROP18*) that encodes a protein (ROP18) released from the secretory organelle known as the rhoptry [26, 27]. In the most recent study in this line of investigation, Etheridge et al. reveal that two other genes (*ROP5*, *ROP17*) and their gene products (ROP5, ROP17) contribute critically to virulence in mice.

These parasite proteins promote virulence by inactivating multiple mouse proteins, including those known as immunity-related GTPases (IRGs) that are expressed at increased levels by cells exposed to interferon-gamma (IFNg) and are involved in killing parasites inside mouse cells [28]. However, the detailed molecular basis for *T. gondii* virulence in humans or even other mouse species does not conform to the pattern in the typical inbred mouse strains used in the laboratory. Humans do not appear to have IRG proteins [29]. So, the ROP proteins that are so crucial in laboratory mice are not the key to virulence in humans. Furthermore, the degrees of virulence of *T. gondii* of Types I, II, and III that are so discernible in mice are not important in the human context. While IFNg is important in humans as in mice for cell-autonomous immunity to *T. gondii*, the key effect of IFNg is increased expression of indoleamine 2,3-dioxygenase (IDO), an enzyme that degrades the amino acid tryptophan. Conversely, IDO is not important in cell-autonomous mouse immunity to *T. gondii*.

The preceding illustrates that virulence in *T. gondii* is both a relational property and a dynamic property that depends on the evolution of the parasite and each host species that it infects. Genetic differences among members of each host species and betweenhost species presumably drive the diversification of the parasite into different lineages with different strategies for optimizing virulence and transmissibility.

16.2.2.2 Transmissibility

A central challenge confronting physicians and other healthcare professionals focused on infectious diseases is tracking the transmission of pathogens both inside and outside of hospitals. One key step in the process of controlling outbreaks of infectious diseases in hospitals is determining whether infections in any particular patient are caused by person-to-person transmission. The traditional approach to addressing this challenge for bacterial infections involves taking account of epidemiological data, assessment of antibiotic sensitivities, and identification of alleles at a limited number (<1 %) of bacterial loci using multi-locus sequence typing (MLST).

A 2012 study [30] demonstrates that whole-genome sequencing (WGS) of infectious isolates from patients involved in a presumed outbreak and covering >95 % of pathogen loci can contribute to determining the transmission network. In this particular study, focused on infection by methicillin-resistant *Staphylococcus aureus* (MRSA), both inpatients and outpatients were likely involved.

This study began in 2011 when the authors identified three simultaneous cases of MRSA carriage in the special care baby unit (SCBU) at a university hospital in

England. Infection-control specialists identified thirteen other SCBU patients with one or more positive screen for MRSA. The team was unable to confirm an outbreak stretching over the relevant time period using conventional approaches. Application of WGS to the relevant pathogen isolates permitted the mapping of a plausible network of infection transmission events.

The WGS provided a number of key insights that might have otherwise remained hidden. First, the team identified a new sequence type (ST) of MRSA, ST2371 that was found to be phylogenetically related to a ST of MRSA, ST22, known to be involved in a high percentage of hospital-associated MRSA infections in the United Kingdom. Thus, it is likely that ST2371 was derived from ST22 but differed (i.e., evolved) from ST22 in having acquired genes encoding an exotoxin, Panton-Valentine leucocidin (PVL), which kills host white cells and has been associated with a dangerous form of pneumonia.

Another important contribution derived from analysis of the WGS data was the demonstration that infants in the SCBU transmitted MRSA to their mothers. There were also transmission events from mothers to other mothers in a postnatal hospital unit and from mothers to their partners outside of the hospital.

The result of applying WGS as part of a prospective longitudinal surveillance program in the SCBU was that a new MRSA infection, after a deep clean of the SCBU and an absence of new SCBU MRSA cases over more than two months, was inferred to be part of the outbreak. The authors therefore screened SCBU staff members for MRSA, and one member of the staff, out of 154, was found to be positive for MRSA. WGS confirmed that the staff member carried the outbreak MRSA, ST2371. These results suggested to the infection-control team that this staff member reintroduced the outbreak-associated strain to at least one patient in the SCBU.

16.2.2.3 Drug Resistance

Perhaps the iconic exemplar of the relevance of evolution to biomedicine and even direct clinical care of individual patients is the development of resistance to antibiotics by bacterial pathogens. The first widely used antibiotic, penicillin, was introduced for mass use in 1943 and over the next seventeen years, its use reduced the incidence of infection-related mortality by more than 93 % compared to 1900 [31]. Nevertheless, already in the 1940s it became clear that some bacteria possessed the capacity to degrade and inactivate penicillin. In fact, it is now clear that antibiotics and antibiotic resistance genes have existed for many millions of years [32].

After the introduction of penicillin, as each subsequent antibiotic was introduced in ensuing decades, resistance followed after varying time intervals but with virtual certainty [31]. The close correlation between antibiotic consumption and the rates of antibiotic resistance in a comparison of European countries [33] supports the inference that chemotherapeutic agents intended to kill bacteria will have the consequential effect of selecting resistant variants that will then increase in frequency. This phenomenon exemplifies the broader phenomenon documented in a

publication by the American Academy of Microbiology that summarizes a 2012 colloquium on the common mechanisms involved in the development of resistance to antibiotics, antivirals, pesticides, herbicides, and anti-cancer drugs [34].

An example of the intricate molecular mechanisms responsible for the development and spread of resistance is provided by the ability of some influenza A viruses to escape treatment with the antiviral agent, oseltamivir. Beginning in 2007, oseltamivir-resistant influenza A viruses containing N1 neuraminidase (NA), a virion surface glycoprotein involved in releasing maturing virions from the surfaces of infected cells, started to increase in frequency. Mutation of histidine to tyrosine at position 274 (H274Y) of the neuraminidase was associated with this resistance [35]. Yet, when this mutation alone was introduced into viruses and the resulting fitness in mice and ferrets, as assessed both *in vitro*, (in tissue culture), and *in vivo*, was reduced. Bloom et al. [36] investigated this apparent paradox suspecting that other mutations in the neuraminidase might permit the H274Y mutation to occur without greatly diminishing fitness. Analysis of a phylogenetic tree for NAs in seasonal H1N1 viruses from 2006 led to identification of two candidate mutations (V234M and R222Q) that could have counteracted the otherwise negative effects of the H274Y mutation on viral fitness among naturally circulating viruses. Additional experiments verified that both of these mutations restored fitness by preventing loss of either total NA enzymatic activity or replication ability.

Structural studies suggest that the molecular mechanism for H274Y-mediated resistance was based on the ability of the tyrosine to limit the motion of the side chain of the glutamic acid at NA position 276 that is necessary for oseltamivir to bind [37, 38]. Understanding the evolution of resistance in biophysical terms at the molecular level offers the possibility of further efforts in drug development to regain therapeutic efficacy.

16.2.2.4 Vaccine Effectiveness

Vaccination is arguably the single greatest contribution to public health and the reduction of morbidity and mortality made by modern medicine [39, 40]. I would not be surprised if the many investigators in this field regarded the elimination of infection by smallpox virus by a worldwide vaccination campaign the single greatest triumph in the application of immunization. One possible consequence of this spectacular achievement may be to regard the implementation of a successful vaccine as the end of the battle to defeat a particular pathogen, but such a perspective takes insufficient account of the potential evolutionary responses of pathogens to such intervention as long as eradication is not achieved. Because many pathogens have non-human reservoirs and may be much more diverse and rapidly evolving than smallpox virus, it is not plausible, as a public health objective, to eliminate most pathogens.

Consider the challenge posed by *Streptococcus pneumoniae* (pneumococcus). This encapsulated bacterial pathogen exists as at least 90 serotypes, with each serotype expressing a distinctive polysaccharide capsule. Although there are serological

cross-reactions among these different capsule structures, antibody-mediated immunity is largely serotype-specific.

As a consequence of this relative serotype specificity in the protective host immune response, the two types of vaccines currently in use clinically to provide immunity to the pneumococcus are both multivalent. The polysaccharide-only vaccine contains 23 different capsular polysaccharides representing the most common serotypes associated with clinical infections. Since this vaccine is relatively ineffective in children less than two years of age, there is also a conjugate vaccine with as many as 13 different capsular polysaccharides independently and covalently attached to a carrier protein.

Studies of the epidemiology of pneumococcal infection after implementation of the conjugate vaccine among young children in various geographic settings have revealed the expected decline in frequency of infection with serotypes represented in the vaccine. Of particular interest for present purposes, infections caused by non-vaccine strains have increased in frequency in some settings [41], a phenomenon called "serotype replacement." Such results suggest that conjugate vaccines can act as reasonably potent agents of selection on the pneumococcal population, thereby shaping the evolutionary trajectory of the pathogen.

The authors of a 2011 [42] study of 240 isolates of a particular multi-drug-resistant strain of pneumococcus from geographic locations on four continents used whole-genome sequencing to address the sources and pace of evolutionary change of this pathogen. Among the interesting findings reported were the following: (1) the majority of genetic variation was due to horizontal gene transfer and recombination between the imported DNA and the host chromosomal DNA as opposed to more classical base substitutions in the chromosomal genes, and (2) loci involved in producing cell surface proteins or capsular polysaccharide were involved more frequently than average loci in such recombination events strongly suggesting selection by the human antibody response. Consistent with the preceding inference, ten of the studied isolates were found to have switched serotypes (determined by the structure of the capsular polysaccharide) to serotypes not represented in the conjugate vaccine available prior to the study.

Such evolution resulting in serotype replacement has implications for vaccine design. In particular, the notion of a universal pneumococcal vaccine based on surface proteins that are relatively conserved in structure among different capsular serotypes may need to include multiple variants of multiple surface proteins encoded in different genomic regions to minimize the chances that one horizontal gene transfer event could eliminate susceptibility to vaccine-elicited antibodies.

16.2.2.5 Chronic Diseases

Beyond the direct relevance of evolution to immunology and microbiology/infectious disease, both immune processes and microbes have been shown in recent years to contribute to the causation of a growing number of medical conditions not previously suspected of involving immune or microbial mechanisms. Examples

include type-2 diabetes [43] and obesity [44]. In 2014, a leading authority on infectious diseases, Martin J. Blaser, published a thorough and accessible discussion of one perspective on the increased risks of allergic, autoimmune, and metabolic disease caused by major perturbations in the relationship that evolved over millennia between the many bacterial species that have historically composed the human microbiome and humans [45]. This book documents the growing body of evidence that substantiates major health effects of heavy antibiotic use by drastically altering the selective pressures on bacteria.

16.3 Conclusion

Evolution on multiple timescales is central to immune responses and immunity to pathogens. Reciprocally, evolutionary phenomena are critical to pathogenetic mechanisms of infectious agents, pathogen transmission, and the development of resistance to medical interventions. So for example, the near-certainty that single-drug therapy for viruses and bacteria will elicit drug resistance has critically informed the design of more effective and more resilient combination chemotherapy regimens. In addition, determining the origins of infectious disease outbreaks and making accurate predictions regarding the qualitative and quantitative features of their spread often rely, respectively, on assessing the phylogenetic relationships of disease isolates and understanding the selection pressures to which specific pathogens are subject. Therefore, a grasp of evolutionary concepts and principles is an essential foundation for both biomedical scientists and clinicians interested in specializing in fields of medicine related to immune system function or interactions with infectious agents.

Glossary

Immunity-related GTPases	A family of proteins in humans, mice, and other mammals that are encoded by genes that can become active in response to the cytokine interferon-gamma. These proteins can catalyze the hydrolysis of guanosine triphosphate to guanosine diphosphate and orthophosphate and can be involved in immunity to vacuolar pathogens by triggering the process of autophagy, which also participates in normal cellular recycling of cellular components. Opsonization—the process by which molecules such as antibodies or proteolytically derived components of the serum proteins that are participants in the complement cascade facilitate the ingestion of bacteria, other microbial pathogens, or other particulates by phagocytes, such as neutrophils, monocytes, macrophages, or dendritic cells.

Pathogen	A microbe or macroscopic parasite that can infect host organisms and can cause cellular dysfunction, tissue damage, and fitness reduction in those hosts.
Phylogenetic relationship	A relationship between species, cells, or genes based on relative temporal proximity to shared common ancestors; thus if we consider three species or three genes, A, B, and C, if A and B shared a more recent common ancestor than either A or B shares with C, A and B are more closely related, phylogenetically, to one another than to C.
Somatic cell competition	A process in which survival and proliferation of non-germ cell body cells, such as Blymphocytes, depends on comparative abilities to acquire a limiting resource, such as critical signals from CD4+ T-cells (so-called helper T-cells) in the case of germinal center Bcells.
Somatic hypermutation	A process affecting Blymphocytes following their activation by antigens in which the portions of immunoglobulin encoding genes that determine the structures of the antibody domains responsible for directly binding to antigen are subjected to an increased rate of mutation. This process typically occurs in germinal centers within lymph nodes or other secondary lymphoid tissues.
Tropism	A characteristic of a virus or other pathogen that pertains to which cell types or tissues of which species can support the replication of that pathogen.
Virulence	Frequently regarded as an attribute of a pathogen pertaining to the extent of debilitation that follows infection; in evolutionary terms, virulence is a relational property attributable to a particular host–pathogen pair that measures the extent to which infection of that host with that pathogen reduces host reproductive fitness.

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