Vertebrate animals possess multiple anti-pathogen defenses. Individual mechanisms usually are differentiated into those that are immunologically adaptive vs. more "primitive" anti-pathogen phenomena described as innate responses. Here I frame defenses used by bacteria against bacteriophages as analogous to these animal immune functions. Included are numerous anti-phage defenses in addition to the adaptive immunity associated with CRISPR/cas systems. As these other anti-pathogen mechanisms are non-adaptive they can be described as making up an "inert" bacterial immunity. This exercise was undertaken in light of the recent excitement over the discovery that CRISPR/cas systems can serve, as noted, as a form of bacterial adaptive immunity. The broader goal, however, is to gain novel insight into bacterial defenses against phages by fitting these mechanisms into considerations of how multicellular organisms also defend themselves against pathogens. This commentary can be viewed in addition as a bid toward integrating these numerous bacterial anti-phage defenses into a more unified immunology.

Nathan suggests that we can "view immunology as the host's participation in the competition between genomes" (p. 173). With bacteria these competing genomes include those associated with bacteriophages as well as the semi-autonomous DNA of plasmids. In this commentary I consider parallels between mechanisms of phage resistance displayed by bacteria and pathogen resistance provided by animal immune systems. The larger goal, toward which this commentary represents only a beginning, is a better integration of considerations of bacterial defenses against phages with study of the myriad defenses all species possess against microbial antagonists. It should be noted in addition that phages, like pathogens in general, possess numerous mechanisms by which they resist or otherwise overcome these defenses. Nonetheless, for the sake of concentrating on the bacterial rather than the phage perspective in this commentary, these phage counter-strategies will not be addressed.

Traditionally, as well as didactically, animal immune-system components have been distinguished into those that are adaptive—a.k.a., specific, acquired, or anticipatory—and those that are not. The latter, also described as innate, are both more evolutionarily primitive than adaptive functions and are the predominant immunity of invertebrate animals as well as of plants. This non-adaptive immunity, often serving even for vertebrate animals as a "first line of host defense," includes a variety of mechanisms that do not change in their specificity over the course of the expressing organism's lifespan, except in terms of changes in gene expression, cell proliferation (or loss), or as a consequence of organism maturation. Innate immunity, in other words, consists of "hard-wired responses" that change in their specificity only as a consequence of undirected germ-line modification. Such relatively fixed protective functions can be of greater utility if they recognize patterns associated with a diversity of potential invaders, that is, if they are relatively non-specific.

Immune functions can be further differentiated into mechanisms that act extracellularly, including at the level of cell membranes, or alternatively intracellularly. Examples of the former include general...
blocks that exist on pathogen penetration into bodies including the barriers associated with mucous or the acidity of gastric juices, i.e., "anatomic and physiologic barriers" (p. 52). An additional aspect of innate immunity is detection of injury,9 a phenomenon also associated with bacteria including in the form of what is known as a phage shock response, which in part is stimulated over the course of filamentous phage adsorption;10 see also Raivio.11

Resistance to pathogens also can result from an absence of factors necessary for pathogens to carry out their life cycles. This can be an absence of surface receptors necessary for viral adsorption or instead difficulty interacting with intracellular molecules that differ among potential host organisms; molecules that can be functionally but not necessarily structurally equivalent. For example, this can be in terms of phage modification of the promotor specificity of a bacterium's RNA polymerase. More strictly defined, however, innate immunity involves the recognition of general patterns seen among organisms, molecules that can be functionally but not necessarily structurally equivalent. For example, to recognize patterns associated with would-be pathogens, such as their lipopolysaccharide or the flagellin making up bacterial flagella, as so too does animal-secreted lysozyme.12 Other pattern-recognition mechanisms—mediated by nucleotide oligomerization domain-like receptors which detect various pathogen patterns such as flagellin as well as mediating the adjuvant activity of alum—in innate immunity is detection of injury,4,9 as discussed in Hyman and Abedon1 as well as Labrie et al.13 The result, in absence of these receptor molecules, is an adsorption resistance by bacteria to phages.

Such envelope-level receptors, as well as various intracellularly located host molecules—such as the NusA protein, the E. coli version of which but not the Salmonella version facilitates antitermination in phase 310—are primary determinants of phage host range. In addition, even bacteria that otherwise are susceptible to a given phage often can mutate to phage resistance by either modifying or eliminating phage-required bacterial factors such as surface receptors.14,15 Organisms in general are similarly innate resistant to most pathogens because of specialization by the latter to the unique molecules associated with specific hosts.16 The result is a relative narrowness particularly of phage host ranges that occur seemingly even in the absence of active bacterial anti-phage defense.4

Like multicellular organisms, bacteria also can display the equivalent of anatomic and physiological barriers to pathogen penetration (encounter blocks) such as extracellular polymeric substances, including capsules. These barriers may be effective, however, only against those proteins or various motifs associated with lipopolysaccharide—to which phages bind in the course of adsorption, i.e., as discussed in Hyman and Abedon1 as well as Labrie et al.13 The result, in absence of these receptor molecules, is an adsorption resistance by bacteria to phages.

Table 1. Bacterial phage-resistance mechanisms and their animal-immune system analogs

| Bacterial mechanism | Description | Immune system analog |
|---------------------|-------------|----------------------|
| Encounter blocks    | Extracellular polymeric substances blocking virion approach to bacterial surfaces, e.g., capsules | Anatomical or physiological barriers, e.g., keratinized skin, mucous, etc. |
| Adsorption resistance (envelope-level resistance) | Absence of necessary receptor molecules on bacterial surfaces, resulting in binding failure | Racial or species immunity |
| Penetration blocks (exclusion; superinfection exclusion) | Blocks on phage movement while in association with host, in this case preventing entrance into host cytoplasm during adsorption | Barrier responses to wounding (e.g., clotting); localization of inflammatory responses |
| Immunity to superinfection (homoinnate immunity) | Recognition of specific phage-associated motifs resulting in blocks on phage replication | Lectin and alternative complement pathways; response to recognition by toll-like receptors |
| Abusive infection   | Killing of phages but at cost of death of individual, phage-exposed bacteria | Apoptosis induced via cell-mediated immunity; action of interferes |
| Restriction-modification | Generic features of organisms are targeted (recognition sequences found in DNA); equivalent host features are protected | Complement, especially alternative pathway; recognition by natural killer cells of absence of class I MHC recognition of absence of Cpg motif methylation |
| Phage growth limitation system | Tagging of phages for elimination by clonally related cells | Osmoporation |

*See Hyman and Abedon1 and Labrie et al.13 for review.
pathogens that do not possess barrier-surmounting adaptations, such as the capsule-degrading depolymerase enzymes produced by some phages.\textsuperscript{4,5,7} The existence of these phage enzymes potentially gives rise to a frequency-dependent selection for rare bacterial adhesion defenses, which in turn may serve as an at least partial explanation for why the chemical structure of bacterial capsules can be highly diverse.\textsuperscript{38}

Pattern recognition, by contrast, involves binding of disruptive self molecules to non-self molecular targets. To be effective, a similarly destructive targeting of self-molecules must be prevented. Strategies for reducing self-targeting come in numerous forms. One means involves a differential masking of patterns that otherwise are associated with both self and non-self. An example of this strategy, as associated with animals, is seen with the alternative complement pathway. Complement factor C3b can bind to both self and non-self surfaces, potentially initiating destructive cascades, but the binding is actively disrupted by self tissues. The result is a reverse recognition of non-self patterns, “It detects markers on host cells and activates on anything that lacks similar markers” (p. 116).\textsuperscript{47} Alternatively, natural killer cells target in part an absence of class I major histocompatibility complex (MHC) molecules, which can be downregulated in tumors as well as by cells infected by certain viruses. Such downregulation occurs for the sake of evading cytotoxic T lymphocytes which, contrasting natural killer cells, target cells that display MHC class I. The result is natural killer cell “recognition of absence of self,”\textsuperscript{22} or “missing self.”\textsuperscript{20} Microbial DNA also can be recognized by animals due to an absence of methylation of CpG motifs (or CpG alkdeguesynucleotides,\textsuperscript{23} that take the form of RRCGYY). These motifs otherwise (p. 123) “are underrepresented in mammalian DNA.”\textsuperscript{19} MHC class I, and CpG motif methylation as well as complement-disrupting molecules, in other words, can be viewed as self-indicating molecular “tags.”

In bacteria a comparable differential-masking function is provided by the common and diverse restriction-modification systems: Recognizable patterns, called restriction enzyme recognition sequences, are found in both host and non-host DNA, such as the palindromic GAATTC of the restriction enzyme, EcoRI. Only non-host DNA is targeted for cleavage however, due to an absence of host factors on that DNA, which are methyl groups supplied by modification enzymes. Indeed, flipping the idea of restriction-modification being driven primarily by the existence of recognition sequences, which of course provides the utility of restriction endonucleases to genetic engineering, it is possible to view this use of recognition sequences instead as a means of limiting the number of targets that must be tagged as self by modification enzymes, i.e., just as presumably is the case for CpG motif methylation described above. In particular, all potential self targets of these enzymes require self modification to keep them from being recognized as “missing self.” Too many potential targets, as would occur given too-short recognition sequences (e.g., two nucleotides long), thus could be both metabolically costly and risky in terms of self being inadvertently left untagged. Alternatively, too few potential targets, resulting from too-long recognition sequences (e.g., ten nucleotides long), can increase the likelihood that foreign DNA will be inadvertently modified prior to its restriction or otherwise evade recognition altogether.\textsuperscript{24,25} Correctly balanced, restriction-modification thus can serve as a relatively metabolically inexpensive general killing mechanism, one that is capable of recognizing patterns potentially associated with a great diversity of targets but which nonetheless is mostly limited in its action to targeting non-self DNA.

Complement factors in animals as well as antibodies can tag pathogens or substances for subsequent elimination, as mediated ultimately via innate immune functions such as phagocytosis. Analogous tagging of phages for subsequent destruction seems to occur in conjunction with the phage growth limitation systems of Streptomyces coelicolor.\textsuperscript{26} Here, phage infection of one cell results in phages that can be destroyed by clonally related bacteria. In particular, a phage burst is allowed by the first infection but not upon subsequent phage infection of related cells. Uninfected cells, once not carrying the equivalent growth limitation system, by contrast can support productive phage infections by these same “second round” phages. The mechanism by which such subsequent phage infections are blocked by the phage growth limitation system may be viewed as a bacterial equivalent to opsonization, i.e., the idea of organisms and materials for elimination from the body environment. This phage growth limitation system thus requires at least two cells to function, the tagging cell which ultimately dies and the second cell which inactivates the resulting phages and ultimately lives.

More generally, bacterial abortive infection systems—which can be likened to the apoptosis seen in multicellular organisms—require more than one cell to be simultaneously useful. The first cell expresses the anti-phage defense but then dies either explicitly because of that defense or instead because phage functions were not blocked early enough to save the cell. The mechanism may be evolutionarily selected, however, only if a second or more inoculated cell, also carrying the abortive infection system allele or alleles, then benefits from the sacrifice of the first cell.\textsuperscript{27} Cellular sacrifice more generally is common in the functioning of animal immunity, such as is seen with the short life spans of neutrophilic leukocytes\textsuperscript{28} or the natural killer- and cytotoxic T lymphocyte-mediated elimination of virus-infected as well as cancerous cells from bodies.

Specific immunity is a characteristic particularly of vertebrate animals and can be described as “induced, highly specific, anticipatory and clonal,” which contrasts with the “nonanticipatory, nonclonal and less specific” nature of innate immunity (p. 13).\textsuperscript{29} “Induced” refers to how specific immunity develops and changes over the course of an animal’s life span. “Anticipatory” means an ability to recognize more patterns than an organism’s lineage is likely to ever see, “…enough receptors in store to cover the entire universe of epitopes, so that for every conceivable epitope there is at least one correspond- ing receptor” (p. 499).\textsuperscript{30} The term “clo- nal,” in turn, refers to a subtle genotypic
systems. Their functioning is exemplified by CRISPR/cas systems, innate immunity.

For bacteria, such “highly specific” immunity is exemplified by CRISPR/cas systems. Their functioning—where CRISPR stands for Clustered Regularly InterSpaced Short Palindromic Repeats—also involves a modification of the genetic endowment of an organism, a process known as adaptation. This adaptation is the product of a molecular mechanism that effects the acquisition of what are known as spacer sequences, DNA sequences that correspond to proto-spacer sequences associated with parasitic DNA.

Mechanisms that serve to deliver this foreign DNA to CRISPR loci may be viewed as analogous in their action to antigen presentation in animals, which contributes to the development of adaptive immunity by making degraded but still potentially recognizable motifs available to helper T lymphocytes. Thus, just as antigens-presenting cells can deliver antigens in the context of MHC proteins to T lymphocytes, so too might various mechanisms provide phage DNA to CRISPR systems. This delivery associated with CRISPR adaptation, though, occurs intracellularly vs. the intercellular antigen presentation seen in animals. Note that prior to this DNA presentation, the phage-infected bacterium must survive, and that survival in at least some instances could be due to the action of bacterial innate phage-resistance functions, though alternatively could instead be associated with phage infections that for various reasons are not metabolically active.4,5

Additional, though more general parallels between bacterial and animal immunity also exist. Immunological layering, for example, is seen in multicellular organisms: “Each phylogenetically new defense mechanism does not replace an evolutionary older one, but supplements it, resulting in a layered structure.” (p. 18). The resulting multiple resistance mechanisms mean that fewer pathogens may be able to evade immune system detection. So too, and presumably for the similar reasons, individual bacteria can possess multiple phage-resistance mechanisms that potentially complement one another. The resulting layering of anti-phage resistance mechanisms can include extracellular blocks, envelope-level resistance mechanisms, various intracellular blocks on both phage infection and phage-mediated killing of bacteria (restriction-modification and CRISPR/Cas systems), and, lastly, abortive infection mechanisms. Included among this layering of multiple resistance mechanisms is a redundant display of similar resistance mechanisms such as the encoding of several restriction-modification systems or instead multiple loci of CRISPR arrays per bacterial genome. These together may serve to increase the likelihood that a bacterial lineage survives phage exposure.

Bacterial mechanisms of phage resistance tend to be individually failable, either with otherwise sensitive phages occasion- ally bypassing specific functions, such as restriction-modification or abortive infection systems, or instead with phages evading bacterial defenses via “escape” or host-range mutations. Abortive infection systems, because they do not protect the viability of individual bacteria even when functioning properly, in fact inherently leave bacteria susceptible to phage-mediated killing. The result is that bacterial populations, in spite of phage resistance mechanisms, often can be overwhelmed given exposure especially to large numbers of phages. This might be viewed equivalently to how most animal pathogens have associated infectious doses, with exposure to lower than infectious doses leading to control by innate immune mechanisms while larger doses potentially overwhelm these same systems. Only those bacteria that have mutated to phage-resistance may survive exposure to phages to which their parent population would be sensitive. Mutations, such as to genes specifying surface molecules, often result in bacterial loss of function, however. For those bacteria possessing CRISPR/cas systems, and therefore adaptive immunity, survival instead may be achieved without such mutational loss. Indeed, an important utility of adaptive immunity, in vertebrate animals, is the achievement of a pathogen-mitigating immune response that is highly targeted within the expressing organism’s genome. Achieving resistance to pathogens through targeted genomic modification presumably is less costly, on average, than resistance that stems instead from genome-wide random mutation.

The parallels between bacteria and multicellular organisms go further than just in terms of their collective need to resist pathogens as competing genomes. Bacteria, in particular, also, can exist in a quasi multicellular form, i.e., as biofilms.7 These biofilms and their constituent microcolonies, like multicellular organisms, however can constitute larger target sizes for acquisition by parasitic or pathogenic organisms. Resulting infections also can give rise to situations where exploitation in one location can lead to exploitation in other locations of the same collection of closely related cells, such as focal infections in animals or, for bacteria, phage penetration into biofilms or microcolonies. The parallels between bacteria and multicellular organisms, like multicellular organisms, however can constitute larger target sizes for acquisition by parasitic or pathogenic organisms. Resulting infections also can give rise to situations where exploitation in one location can lead to exploitation in other locations of the same collection of closely related cells, such as focal infections in animals or, for bacteria, phage penetration into biofilms or microcolonies.7 It is likely that for clonal organisms to take up multicellular or colonial lifestyles, given this perhaps inherent potential for increased vulnerability to existing pathogens, they must first display greater levels of resistance or immunity than can be required for the success of smaller as well as more-dispersed single-celled organisms. Bacterial microcolonies thus might serve as models for the study of evolutionary transitions from a unicellular to more colonial or multicellular existence, including in terms of the development of strategies of anti-pathogen immunity.

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