Graduate Writing Advice – Peter Southern

Comments written in response to the common student question: "What can I do to improve the quality of my written answers?"

The evaluation of any and all writing is highly subjective but there are certain standards and conventions that apply widely in scientific writing and you need to be aware of these expectations as you work on graduate school writing assignments.

High quality written answers meet the following criteria:

1) Clear outline and logical flow of ideas
2) High factual content
3) Impersonal and concise writing style
4) Careful focus on the specific topic of the question.

You might try to argue that if the instructor is the target audience for your paper then you can omit facts because it is reasonable to assume that the person setting the assignment should be very familiar with all of the facts underlying their own questions. In reality, you are writing to convince the instructor that you have a clear understanding of the complexities of the question and that you have acquired a body of basic information relating to the key components that comprise a comprehensive answer.

Suggested Planning Strategy

Decide exactly what you think is the primary focus of the assigned question. Start this evaluation as soon as possible.

Decide if there are secondary issues that should be addressed in writing a complete answer. Almost every type of written answer will be improved by inclusion of concise and relevant background information.

Do not paraphrase or simplify the assigned questions. This frequently results in the failure to recognize an essential component of a complete answer and consequent loss of a significant portion of the available credit.

Ask yourself many questions and make connections between your questions to establish a clear outline and structure for your answer.

Do not perform endless literature searches in pursuit of one “ideal paper” that will provide all the information you are seeking in a single source. The best questions will require you to distill information from different sources to create a concise and focused answer that successfully links all of the components together. Assemble a list of key facts and statements and consider the most logical scheme to establish connections between various pieces of information.
Specific Writing Suggestions

Use a new paragraph to make one major point, or a series of interrelated points that are not discussed extensively.

Express your thoughts such that there is a clear sense of continuity between the end of one sentence and the beginning of the next sentence.

Try to write individual sentences that still make a clear point when isolated from the remainder of the paragraph. With this goal in mind, minimize the use of pronouns.

You are not writing for TIME Magazine so avoid dramatic exaggeration and the expression of personal feelings – either your own feelings or the perceived feelings of the central topic of your paper. Scientific writing is predominantly impersonal and passive although there some indications that this may be changing. Most importantly, settle on the style you want to use and then write consistently in this style.

Proof reading is an essential component in the completion of a strong written answer.

Proof reading can help you identify:
1) Gaps in the flow of your arguments and explanations
2) Excessive repetition. There is a fine balance between repetition for emphasis and repetition that arises from imprecise thinking and poor organization
3) Awkward and ambiguous statements
4) Typographical errors

Ideally, you should aim to finish your writing so that careful proof reading can be performed the day before the paper is due. Read your text aloud. If you have trouble reaching the end of a sentence without gasping for breath then that sentence is too long. Similarly, the sentence is too long if you have lost track of the subject, or the major point being made, before you reach the end of the sentence.

I have included examples of high quality graduate student writing to provide a sense of what the expectations are. These are unedited student answers that were written by first or second year students, in response to the stated questions.

Note that most answers will require you to include 5-10 primary literature citations that you have found to be especially useful. Textbooks are web sites DO NOT constitute appropriate literature citations to support your written answers – you must list references from the primary literature. Textbooks and web sites can be valuable resources for a first layer of background information and as a guide for specific literature searches. Any standard format to list the references is acceptable – the simplest approach is to include numbers in parentheses in the text and list the references in numerical order at the end of your text.
Sample Assignment I

This assignment comprises one question with two interrelated components, A and B. Make sure that your answers address the specific issues raised in both A and B. Answers should be about 4-6 pages of double-spaced text (10pt or 12pt font) and should include a listing of key references. Answers will be graded out of 40 points.

Answers that are judged to be adequate will receive approximately 70% credit. Answers that are significantly better than adequate will receive most/all of the credit. Answers that are less than adequate will be scored accordingly.

Q1A) Explain how analysis of the genomic sequence of *Mycobacterium tuberculosis* has contributed to a more complete understanding of microbiological properties of this pathogenic bacterium.

Q1B) Outline the principal differences between *Mycobacterium tuberculosis* and *Mycobacterium leprae*, as revealed by genomic sequence comparisons that may account for the observed differences in pathogenic potential for these two mycobacteria.

Tuberculosis is a worldwide health problem affecting approximately eight millions new individuals per year around the world.¹ The sequencing of causative agent of tuberculosis, *Mycobacterium tuberculosis*, was complete in 1998 and the sequence information has been used to better understand the organism. According to the genomic sequence, the size of *M. tuberculosis* is 4.4 Mb and contains approximately 4000 genes. Analyzing the genes of *M. tuberculosis* has revealed interesting characteristics of the organism.

One of the ways the analysis of the complete genome of *M. tuberculosis* has helped to understand the organism is by revealing the existence of genes of known function. One such example is in *M. tuberculosis* metabolism. *M. tuberculosis* has traditionally been thought of as an aerobic organism, but the genomic sequence has shown that *M. tuberculosis* may also have anaerobic character. The presence of nitrate reductase and fumarate reductase in *M. tuberculosis* genome strongly suggests anaerobic
character. Nitrate can be used as an alternative terminal electron acceptor instead of oxygen and fumarate reductase allows the TCA cycle to function in anaerobic conditions.\(^2\) To investigate the role of nitrate reductase, Weber et al mutated the gene for nitrate reductase in \textit{M. bovis} BCG which is homologous to the nitrate reductase gene in \textit{M. tuberculosis}.\(^3\) Weber et al showed that both wild type \textit{M. bovis} BCG and the mutant lacking nitrate reductase were able to grow equally well in aerobic condition. However, when infected in immunodeficient mice, wild type \textit{M. bovis} BCG grew large granulomas while the \textit{M. bovis} BCG lacking nitrate reductase grew smaller granulomas. Furthermore, all mice infected with wild type \textit{M. bovis} BCG showed clinical signs of infection within 50 days while mice infected with \textit{M. bovis} BCG lacking the nitrate reductase showed no clinical signs even after 200 days. We can surmise that the anaerobic conditions of granuloma formation may necessitate the use of anaerobic mechanism of energy production in \textit{M. tuberculosis} for the organism to achieve high virulence.

Another way that the analysis of the complete genome of \textit{M. tuberculosis} has helped to understand the organism is the discovery of new genes for which there is no known function. Once the sequence of a gene is known, the function can be deduced via knock out or over expression of the gene product. An example of genes of unknown function in \textit{M. tuberculosis} that has sparked interest are the two gene families, PE and PPEs.\(^4\) The PE and PPE families both contain conserved N-terminal domain with Pro-Glu motif in PE family and Pro-Pro-Glu in the PPE family. PE and PPE families of genes can be further divided into subfamilies depending on the C-terminal sequence. The interest in PE and PPE family stems from the fact that they represent a large (8%) part of
the genome. One of the possible roles of PE family of genes may be related to proliferation in macrophages. Triccas et al showed that a gene from a subfamily of PE, PE-PGRS, is up regulated when *M. tuberculosis* is phagacytosed by a macrophage.\(^5\)

Following this observation, Ramakrishnan et al showed that the inactivation of the PE-PGRS homologue in *M. marinum* lead to the inability of *M. marinum* to replicate in macrophages.\(^6\) Another possible role of the highly variable sequences of PE and PPE subfamilies is antigen variability. By inserting sequences in the variable regions of PPE subfamilies, the size and structure of the protein will change. Further, there is evidence that some of the variable PPE subfamilies are surface proteins and may act as antigens. The strategy of varying the surface antigen to blunt host immune response is used by other bacteria, like *Neisseria*, as well and may be contribute to the virulence of *M. tuberculosis*.\(^7\)

Access to the complete genome of *M. tuberculosis* has enabled the researchers to utilize comparative genomics to study *M. tuberculosis*. When the genome of *M. tuberculosis* is compared to closely related subspecies that cause tuberculosis, there is 99.9% similarity in the sequence. Despite near perfect sequence identity, *M. tuberculosis* and subspecies have variable phenotypes of which the most notable being species tropism. The genome sequences of *M. tuberculosis* H37Rv and *M. bovis* BCG were compared and deletions were found in *M. bovis* BCG named RD1 through RD16. Study of the deleted sites has helped to characterize *M. tuberculosis*. For example, RD1 region contains 8 genes of which most belong to the ESAT6 gene cluster. ESAT6 is an antigen recognized by the host immune system and stimulates the immune response.\(^8\) The genes in the deleted region, RD1, are present in the virulent *M. bovis* and *M. tuberculosis* while
absent in the non-virulent *M. bovis* BCG. Therefor, RD1 region may play a role in
virulence. Another interesting deleted region is RD7. RD7 contains the mce3 operon
and codes for an invasin-like protein in *M. tuberculosis*. Arruda et al was able to show
that *E. coli* expressing mce gene was able to invade HeLa cells. Since RD7 is missing in
some of the *M. tuberculosis* subfamilies that are not virulent in humans, it is possible that
genes in the RD7 region may play a role in invading human tissue.

We can also use comparative genomics to study *M. tuberculosis* and *M. leprae*.
*M. leprae* is 3.3 Mb in size and only 49.5% of the genome is a coding sequence compared
to *M. tuberculosis* which is 4.4 Mb in size with 90.8% of the genome being a coding
sequence. The number of active genes in *M. leprae* is 1604 compared to over 4000 genes
in *M. tuberculosis*. So, what is the rest of *M. leprae* genome? About 27% of the *M.
leprae* genome corresponds to 1116 inactive genes that have a functional counterpart in
*M. tuberculosis*. The last 23.5% of *M. leprae*’s genome are non-coding. Of the 1604
active genes in *M. leprae*, 1439 genes are in common with *M. tuberculosis* leaving just
165 genes that is unique to *M. leprae* compared to *M. tuberculosis*. The massive loss of
genes, or reductive evolution, in *M. leprae* is likely due to deletions and mutations of
genes not necessary once the organism became an intracellular parasite. In effect, the
reduced gene load of *M. leprae* may represents the minimal coding sequence needed for
mycobacterium survival.

Analyzing which genes are mutated or deleted in *M. leprae* reveal interesting
results. One interesting gene is the katG gene. KatG codes a catalase/peroxidase in *M.
tuberculosis* which helps to neutralize oxygen radical and activates the antibiotic
isoniazid. KatG plays a role in protecting the mycobacterium from the macrophage’s
use of oxidative burst to kill the organism. However, the catalase inadvertently activates the antibiotic isoniazid which may explain why *M. tuberculosis* is susceptible to isoniazid while *M. leprae* which has the mutated KatG is not sensitive to the antibiotic. Another interesting loss in *M. leprae* genome is the genes involved in anaerobic metabolism previously discuss, namely nitrate reductase and fumarate reductase. Since the environment in which both *M. leprae* and *M. tuberculosis* is oxygen limited, it is interesting that *M. leprae* has lost these genes. This loss of anaerobic metabolism may explain the very slow doubling time of *M. leprae*. Another metabolic difference between *M. leprae* and *M. tuberculosis* is the number of active gene in lipid metabolism. In *M. tuberculosis* there are 225 genes involved in lipid metabolism including 22 lipases while *M. leprae* has reduced the number genes to 14 with only 2 lipases. The impressive change in number of lipid metabolism genes may reflect a limitation in number of lipid sources which *M. leprae* can utilize for energy. The limited energy source may explain the difficulty in efforts to culture *M. leprae*.

The complete sequence of *M. tuberculosis* and related organisms, such as *M. leprae*, has revolutionized the way we study mycobacterium. The discovery of genes of known function, of unknown function, and the ability to perform comparative genomics has revealed wealth of information. Although many of the findings were not included in this paper, the advantage of a complete genome is apparent.
Bibliography

Sample Assignment II

Answers should be 3-5 pages of double-spaced text (10pt font). Please include 5-10 references that you found to be particularly valuable as you reviewed information to prepare your written answers. General textbooks are not usually considered appropriate for use as literature citations in scientific writing at this level.

Answers that are judged to be adequate will receive 60-65% credit. Answers that are significantly better than adequate will receive correspondingly more credit. Answers that are judged to be less than adequate will be scored appropriately.

With this question, there are not necessarily “right” or “wrong” answers but it is entirely predictable that some answers will be better than others. A large part of the challenge for this writing assignment is for you to determine what are the most important topics to be presented and how you should best organize your answer. Accordingly, it is extremely unlikely that any information will be provided to clarify the question further.

One question, worth 35 points:

Review the current understanding of pathogenic events in *Helicobacter pylori* infections.

The hostile acidic environment of the stomach protects us from most ingested infectious agents. Yet the gram-negative bacterium *Helicobacter pylori* has evolved not only to survive in the stomach, but to colonize it. *H. pylori* has acquired adaptations for surviving the gastric mucosa and mechanisms to downregulate the immune response in order to establish decades-long infections. Half the world’s population is colonized, but the clinical manifestations of infection differ dramatically, from asymptomatic microscopic gastritis to cancer. Why many who are exposed don’t become infected, and why only some who do progress to disease is unknown. *H. pylori* status remains the greatest risk factor for peptic ulcer disease, gastric adenocarcinoma, and MALT lymphoma.

Epidemiologic data suggest the mode of transmission may be oral-oral or fecal-oral. Infection is more prevalent in developing nations and in lower socioeconomic classes in developed nations, pointing to poor living conditions, personal hygiene, and overcrowding as risk factors. No animal or environmental reservoir has been identified. Genetic factors also play a part in infection, as first-degree relatives of infected children are more likely to be infected, and monozygotic twins have a higher concordance of ulcer disease as opposed to dizygotic twins. People who work in endoscopy suites are also more likely to be infected, implicating person-to-person transmission.
Infection begins after ingestion. The microaerophilic organism can survive in the low levels of oxygen of the digestive tract. It uses a urease enzyme to split urea into ammonia, buffering the acidic environment. Though H. pylori strains show extreme genetic diversity, urease is highly conserved, attesting to its importance in infection. Urease-negative isolates are unable to colonize mice. Once the local environment has been neutralized, the bacteria use 2-6 flagella to propel themselves through the thick gel layer that protects gastric mucosal cells from the acid. The bacteria cluster in the mucus layer directly overlaying the epithelial cells, with around 20% adhering to gastric epithelial cells. This intimate contact allows for acquisition of nutrients as the cells die, resistance to peristalsis or shedding of the mucus layer, and toxin secretion into epithelial cells. The bacteria prefer to bind to the upper portion of gastric glands, and the deep glandular regions have been shown to contain a mucin component that interferes with the biosynthesis of a major portion of the H. pylori cell wall. Adhesion is mediated through at least two outer membrane proteins. Blood-group antigen binding protein, BabA, binds Lewis B (LeB), a fucosylated antigen on red blood cells and the gastrointestinal mucosa. However, BabA mutants can still adhere, and blocking of LeB does not inhibit binding. This led to the identification of a second adhesin, SabA, that binds sialyl-Lewis X (sialyl-LeX). This ligand is not expressed in healthy gastric epithelium, but is induced during inflammation. It normally binds selectins on lymphocytes, allowing them to infiltrate through the endothelium into inflamed areas. Expression of a selectin mimic helps explain how inflammation benefits the bacteria, and how they can persist in inflamed tissues for long periods of time.

To establish a chronic infection, H. pylori must balance carefully the amount of damage it causes and the extent of immune response it activates. Too much mucosal damage eliminates its niche, and too exuberant of an immune response will eradicate the infection. The bacteria have amassed an impressive repertoire of tools to strike this fine balance. For example, most bacterial infections activate toll-like-receptors (TLRs) that recognize bacterial molecules, such as LPS and peptidoglycan, and induce inflammation. However, H. pylori LPS is a weak activator of this response, and its flagellin protein similarly fails to productively engage TLR-5. These adaptations prevent an overwhelming anti-bacterial response.

A number of virulence factors have been described. VacA is a secreted toxin that forms large intracellular vacuoles in epithelial cells. It binds to tyrosine phosphatase receptor type Z (Ptprz) and causes
Ulceration. Oral administration of VacA to mice results in gastritis, but not in ptprz<sup>−</sup>− animals. The endogenous ligand pleiotrophin also causes gastritis, but only in ptprz<sup>+/−</sup> animals. In vitro studies show VacA mediates cell detachment, which could contribute to ulcer formation. In addition, the toxin causes apoptosis of epithelial cells through release of cytochrome c. It prevents the fusion of phagosomes with lysosomes after phagocytosis of H. pylori by macrophages, where the bacteria can survive for up to 24 hours. VacA creates pores in epithelial cells, permeabilizing them to urea and allowing a flux of this important molecule into the lumen, supplying the bacteria with the substrate with which to create ammonia. The toxin also has been shown to increase thrombin expression, which may be protective for the mucosa. This activity may limit the damage caused by the bacteria and preserve its environment. Almost all strains produce the toxin, but its activity varies. S1 strains show higher activity and correspondingly more severe gastritis and higher risk for peptic ulcer disease and gastric cancer. The less virulent s2 strains produce VacA that is defective in membrane insertion, apoptosis induction, and vacuole formation.

A less well-studied factor is encoded by iceA. The exact function of the protein is not known, but it is found in approximately 25% of US isolates, which, perhaps coincidentally, is also the percentage of infected patients who progress to ulcer disease and/or cancer. The presence of iceA is associated with vacAs1, severe inflammation, and increased interleukin-8 (IL-8), a proinflammatory cytokine.

IceA is also mostly found in strains positive for the cag pathogenicity island. Sixty percent of US strains carry this group of 27 genes that, like the presence of VacA, significantly increase the risk for severe gastritis, ulcers, and distal gastric cancer. The island encodes CagA and a type IV secretion system, allowing the CagA protein to be directly injected into gastric epithelial cells. Once inside, it is phosphorylated by src protein tyrosine kinase and has an overall mitogenic effect, driving proliferation and motility. This classic deregulation of proliferation is thought to be one mechanism of H. pylori oncogenicity. The bacteria show tropism for tight junctions, and CagA associates with tight junction proteins, disrupting the structure and resulting in monolayer leakage and loss of polarity. It is thought that the loss of polarity leads not only to ulceration, but to dysregulated growth and dysplasia. All of these activities also result in increased apoptosis of the epithelial cells. CagA forms a complex that phosphorylates and inactivates src family kinases, leading to its own downregulation (although some CagA
activities are phosphorylation-independent). This limits toxicity and may be another mechanism for long-term survival.

Such survival depends on creating enough inflammation to upregulate molecules for adhesion and to disrupt the epithelial architecture, creating a favorable environment and making nutrients available, but without damaging the bacteria. The inflammatory response starts with the reaction of gastric epithelial cells. Upon infection, they produce IL-8, which is chemotactic for neutrophils and other immune cells. Levels of IL-8 have been shown to correlate directly with severity of gastritis. Another cag-encoded protein, CagE, is involved in IL-8 expression, and inactivation of this locus attenuates gastritis. IL-8 is also regulated by oipA, and strains positive for oipA create significantly more inflammation. Induction of IL-8 is mediated through ligation of TLR-2 and TLR-5, and subsequent activation of p38. It can also be induced through TLR-independent mechanisms, involving ERK1/2 and JNK kinase activation.

The resulting inflammatory infiltrate is characteristic of chronic gastritis. However, it does not accomplish bacterial killing, even though H. pylori is a potent inducer of the neutrophil respiratory burst. After nonopsonized bacteria are engulfed by neutrophils, they disrupt the targeting of NADPH oxidase so that superoxide anions are released not into the phagosome but extracellularly, sparing the bacteria and further injuring the epithelial cells. This activity is dependent on SabA and VacA, but not BabA. H. pylori have also been shown to survive in vacuoles of epithelial cell lines, suggesting another way they might escape killing.

H. pylori can also damage the mucosa through its detrimental effect on gastric mucin synthesis. Mucin provides protection for the gastric epithelial cells from the acid and proteases in the stomach lumen. H. pylori LPS mediates endothelin-1 upregulation and binding to the G protein-coupled ET(A) receptor, resulting in decreased mucin production and, presumably, more damage to epithelial cells.

The mucosal damage is also caused by T cells, as T cell-deficient SCID or RAG\(^{-}\) mice show less mucosal injury while B-cell deficient mice show the same pathology as wild-type. The offending T cells are likely Th1 cells, induced by the high levels of IL-12. B6 mice, which have a tendency to mount Th1-type responses, show more gastritis than the Th2-skewed Balb/c mice. Transfer of Th2 cells or coinfection with Th2-inducing helminths attenuates gastritis. It is tempting to speculate that the high prevalence of
parasitic infections in developing countries may help decrease gastritis due to the simultaneously high prevalence of H. pylori infection.

In addition to IL-8, epithelial cells produce IL-1β, IL-2, IL6, and TNF-α, all pro-inflammatory cytokines, in response to infection. IFN-γ also plays a role, as blockade reduces gastritis. Patients with polymorphisms favoring increased IL-1β or TNF-α expression have higher risks for gastric cancer, as do patients with reduced IL-10, an immune suppressive cytokine. The severe inflammation in these patients leads to destruction of acid-producing cells. Without enough acid, the patient suffers from hypochlorhydria, which is followed by atrophic gastritis, intestinal metaplasia, in which gastric cells take on the morphology of intestinal epithelium, and, ultimately, higher risk for adenocarcinoma. Contrary to popular belief, it is the reduction of acid, and not its overproduction, that leads to peptic disorders. Interestingly, H. pylori infection significantly reduces the risk of gastroesophageal reflux disease, Barrett’s esophagus, and esophageal cancer, all thought to be due to gastric acid contacting the esophageal lining.

Highly increased inflammation is actually beneficial to the host, as shown by mice deficient in IL-10, which spontaneously clear infection and show more severe gastritis than wild-type mice. This seems biologically relevant, as infection induces dendritic cells to express both IL-12 and IL-10, which may modulate the response. Nude mice that receive T cells that have been depleted of CD25+ regulatory cells also show more intense inflammation and harbor reduced bacterial loads. H. pylori may activate endogenous Tregs to help it persist.

H. pylori has also evolved ways to evade the adaptive immune response. VacA can inhibit T cell proliferation through an unclear mechanism. The bacteria themselves or protein extracts of culture supernatants also inhibit lymphocyte proliferation. This activity, distinct from VacA, is independent of known virulence factors and found in a 30-60 kDa protein that blocks S-phase entry and arrests T cells in G1. Cell surface molecules can also be varied, leading to antigenic variation that defies immune clearance. Antibodies are not protective. The enzyme arginase affects both innate and adaptive responses, by siphoning L-arginine away from iNOS, limiting NO production by macrophages, and also by limiting T cell proliferation through reduction of TCR CD3ξ-chain expression.

H. pylori is therefore uniquely suited to the inhospitable environment of the human stomach. It creates controlled inflammation without detriment to itself and without productive activation of the immune
response. These strategies allow H. pylori to establish long-lived infections and, in 20% of infected people, cause significant morbidity and mortality. Understanding the events in pathogenesis can provide molecular targets for rational drug design and strategies for vaccination. As gastric cancer is a leading cause of cancer death in some parts of the world, further study could lead to a considerable improvement in global health.


November 2005 Southern Writing Assignment III

Answers should be 4-6 pages of double-spaced text (10pt font). Please include 10-15 references that you found to be particularly valuable as you reviewed information to prepare your written answers. General textbooks are not usually considered appropriate for use as literature citations in scientific writing at this level.

Answers that are judged to be adequate will receive 60-65% credit. Answers that are significantly better than adequate will receive correspondingly more credit. Answers that are judged to be less than adequate will be scored appropriately.

With this question, there are not necessarily “right” or “wrong” answers but it is entirely predictable that some answers will be better than others. A large part of the challenge for this writing assignment is for you to determine what are the most important topics to be presented and how you should best organize your answer. Accordingly, it is extremely unlikely that any information will be provided to clarify the question further.

One question, worth 65 points:

Why vaccinate?

Develop your answer with specific reference to the current vaccine situation for influenza virus, human papilloma virus type 16 (HPV 16) and hepatitis C virus (HCV). You may also include examples of other vaccines or microbes that support your arguments.

Why Vaccinate?

In 1796, Edward Jenner discovered that exposing a person to cowpox, or vaccinia, protected that person against smallpox, an often deadly disease. He called his procedure vaccination. Indeed, we still use this term today to describe the inoculation of an individual with weakened or attenuated strains of disease-causing agents in order to provide protection from disease.

In 2005, it is generally accepted that vaccination is a safe, efficient, and cost-effective strategy to protect against viral infections. A prime example is the widespread support for the annual flu shot. Business-owners provide employees with influenza vaccinations in order to avoid decreased productivity. Moreover, insurance companies cover the cost of the flu shot at the doctor’s office. Still, there is
reluctance to getting vaccinated. For example, rumors spread that it is possible to become ill from the flu shot. Some say that they have never had the shot, so why start now?

In an attempt to answer this very important question, let us take a look at the current vaccine situations for three important viruses, beginning with influenza virus, and following with human papilloma virus and hepatitis C virus.

Acute respiratory infections are the leading cause of acute illness throughout the world. They are responsible for almost 4 million deaths per year. In the USA alone, there are 25 to 50 million cases per year, leading to 150,000 hospitalizations and 30,000-40,000 deaths. The main infectious agent causing these infections is influenza virus. It causes acute infection, characterized by severe disease that develops quickly and induces a vigorous immune response that works to get control of the infection.

Phylogenetically, influenza virus belongs to the family Orthomyxoviridae, which is comprised of three genera: Influenzavirus A, Influenzavirus B, and Influenzavirus C. Influenza A has many subtypes based on the antigenicity of its major envelope glycoproteins, hemagglutinin (HA) and neuraminidase (NA).

Influenza viruses infect host cells by binding to receptors on epithelial cell surfaces via HA. After replication within the host cell, newly synthesized viruses are released by the action of NA. Anti-HA antibodies (Abs) produced by the host’s humoral immune response neutralize the infectivity of the virus, and anti-NA Abs prevent the release of viruses from host cells. Since it takes approximately seven days for the host to develop acquired immunity, and symptoms begin within one to three days, disease can be prevented only through previous exposure by natural infection or vaccination.

The annual flu shot provides exposure to certain strains of influenza virus, but the properties of the virus make this a difficult situation. For one, influenza virus is subject to antigenic drift, or the accumulation of point mutations in genes encoding HA and NA, which results in the constant emergence of novel virus strains of which there is little or no pre-existing immunity in the population. Antigenic drift is responsible for almost yearly influenza epidemics and greatly influences the effectiveness of influenza vaccines. The ability of influenza viruses to rapidly mutate is partly due to their RNA genomes: RNA polymerases lack the ability to proofread and therefore replication errors occur on the order of one in every 10,000 bases per replication cycle, which is significant for the 13,500-nucleotide influenza viral genome.
In addition, the genome is segmented, allowing for mutations to occur on different genes by reassortment. Studies conducted by the World Health Organization use sequence evaluation and hemagglutination inhibition cross-neutralization to predict what the prevailing strain will be for the upcoming year, and attempt to match the vaccine to the challenge strain.

However, even careful analysis by the WHO cannot account for another characteristic of influenza viruses, antigenic shift. The segmented nature of the genome allows for the virus to acquire genes from other animal influenza viruses, leading to a completely new glycoprotein subtype. If the recombined virus is able to infect humans and spread from human to human, a pandemic (worldwide epidemic) may occur. Three pandemics occurred in the last century, in 1918, 1957, and 1968. The avian influenza virus, which has an avian virus HA glycoprotein, has pandemic potential. The most recent outbreak has infected over 70 people to date. If a pandemic were to occur in 2005 of similar magnitude to that of 1968, the economic cost is estimated to be US$ 167 billion (including only direct medical costs and lost productivity as a result of disease and deaths).

Although rapid mutation rates are a conundrum in the development of vaccines against influenza virus, reverse genetics enables scientists to develop vaccines against any potential virus strain. Several influenza subunit or DNA vaccine candidates are being developed. There is hope that broad spectrum immunity against multiple strains will be induced by new vaccines.

For the time being, influenza vaccines aim to mimic natural infection by target viruses. Currently utilized inactivated vaccines made from detergent-split influenza virus grown in the allantoic cavity of embryonated chicken eggs are effective in preventing illness and have a high benefit-to-cost ratio. However, they do not produce heterosubtypic immunity (they do not protect against drift viruses).

In contrast to inactivated vaccines, cold-adapted live attenuated influenza virus vaccines are in Phase III clinical trials. These vaccines more closely mimic natural infection – by triggering mucosal immune responses and inducing a cell-mediated immunity – and thus provide significantly better cross-protection against infection with a broad spectrum of viruses that is longer-lasting compared to conventional inactivated vaccines (protection rate of 92%, including antigenic variants). In addition, live attenuated vaccines are safe, effective, and have remarkable genetic stability. They are now licensed in the USA for vaccination of individuals 5 to 49 years of age.
Even though influenza vaccines are far from perfected, studies have indicated that mass-vaccination of school-aged children is related to reduced rates of respiratory illness in all age groups, and thus favorable effects on influenza epidemics.

Unlike most influenza types, which cause acute, self-limited infections, human papilloma virus (HPV) causes chronic infections in over 30% of all cases. This means that the initial, acute infection is not severe enough to provoke a strong enough immune response to clear the infection. The viral load is reduced, but the virus is maintained within the host, where it evades or even inhibits the immune system, all the while causing low levels of insult. Viruses such as HPV thus present new challenges in vaccine development.

HPV infection is the central causal agent for cervical cancer. HPV 16, which is a high-risk type and the most common type in nearly all countries, is present in more than 99% of cervical cancers and high-grade squamous intraepithelial lesions. In developed countries, Pap cytology screening has reduced the incidence of cervical cancer morbidity and mortality during the last 50 years. However, these data do not account for developing countries, where such screening is expensive and not widely used. Furthermore, screening results are not always accurate: a critical limitation is the test’s high-false negative rate. False-negative diagnoses have important medical, financial, and legal implications. For instance, they are the most frequent reasons for medical malpractice litigation in North America.

Cervical cancer is second only to breast cancer in overall disease burden for women throughout the world. It is the most frequent infectious-related cancer in the world. Every year, 500,000 deaths from cervical cancer occur. Three-fourths of these deaths take place in developing countries, where women are the primary educators and role models for their children. The premature loss of these mothers has a severely negative impact on the social structure of local communities. It is true that most sexually active women will acquire HPV at some point during their lifetime.

The challenge in vaccine development of HPV begins with its ability to cause chronic infection. HPV penetrates the suprabasal epithelium of the human cervix and represses the transcription of its late genes, L1 and L2, which are powerfully immunogenic capsid genes. HPV also lacks cytopathic features and highly immunogenic double-stranded RNA (HPV consists of a circular, double-stranded DNA of approximately 8000 base pairs, enclosed in a non-enveloped nucleocapsid), thus blocking innate or
acquired immune responses seen in other viral infections. The host is ignorant to the virus’ presence, permitting the persistence of the replicative life cycle and leading to chronic infection.

Early non-structural proteins, mainly E6 and E7, also have negative immunomodulatory effects. E6 inhibits epithelial cell interaction with dendritic cells, which are vital in the body’s defense against infectious agents and cancer. Furthermore, E6 and E7 block interferon 1 production in infected cells and also inhibit the activity of the important chemoattractant protein 1.

Knowledge of the pathogenesis of HPV has guided researchers to approach vaccination in two different ways. For one, prophylactic vaccines aim at reinforcing natural immunity against invasion of HPV in tissues that have not yet met these antigens to prevent primary infection. L1 major capsid protein (and L2 capsid protein) is the target for neutralizing antibody production. These Abs protect against transmission and acquisition of HPV infection. By using self-assembled, DNA-free virus-like particles (VLPs) generated from fusion proteins of recombinant L1 or L1/L2 capsids, a neutralizing Ab response can be induced. The VLPs cannot replicate, making them noninfectious and without oncogenic risk. Pentamers of the most immunogenic sequences of L1 enhance the magnitude and prolong the response against HVP.

Experiments with animal models showed that prophylactic vaccines protected animals against HPV infection. Passive immunization of animals with serum from vaccinated animals also prevented infection. Two different studies have found vaccine efficiency to be 100% in preventing acquisition of persistent HPV infection. Clinical trials are currently underway for an HPV-16 vaccine. So far, there have been no vaccine-related serious adverse effects. The vaccine appears safe and effective. As such, there is hope of preventing widespread HPV-associated cervical cancer using this VLP-based prophylactic vaccine.

In addition to developing prophylactic vaccines for HPV, researchers are also working to find a therapeutic vaccine that would induce a stronger immune response (beyond the maintenance level) in those already infected with HPV. A therapeutic vaccine would aim to prevent progression of the infection from low-grade squamous intraepithelial lesions to high-grade squamous intraepithelial lesions, to achieve regression of cervical intraepithelial neoplasia (CIN) or condylomata, or to eradicate residual cervical cancer. One way to achieve these effects is by inducing cytotoxic T lymphocytes (CTLs) to E6 and/or E7, which are oncoproteins responsible for malignant transformation and immortalization. Protein products of
E6 and E7 interfere with normal function of tumor suppressor genes p53 and retinoblastoma protein (pRB). Inducing antigen-specific type 1 T cell responses against E6 and E7 may result in vaccine-induced regression of pre-cancerous lesions or remission of advanced cervical cancer.

Because of the long incubation period of HPV, merely preventing future HPV infection fails to take into account those who are already infected. Therefore, therapeutic vaccines are necessary to bridge the temporal gap by attacking already established HPV infections. Therapeutic vaccines will supplement or even circumvent current treatments for HPV, which rarely cure the infections of often young patients with recurrent or persisting CIN. Neither surgery nor combined radiochemotherapy are of much help to these individuals.

Another virus that urgently calls for a vaccine is hepatitis C virus (HCV). HCV causes chronic infection in 55-85% of cases. It is the most common cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) in developed countries. An estimated 170 million people are infected worldwide. In the USA, HCV is the most common chronic bloodborne infection.

HCV is a positive-stranded RNA virus with a genome consisting of approximately 9400 nucleotides. HCV belongs to the Flaviviridae family. It demonstrates significant heterogeneity: at least six major genotypes are found in various geographic areas, and there are at least 70 subtypes. In addition, there is considerable quasispecies diversity in individual HCV isolates. Despite such diversity, all genotypes encode for the E1 and E2 proteins, which are glycosylated and form the envelope of the virus.

Upon infection with HCV, both humoral and cellular immune responses are important for the host to escape chronic infection, however cellular responses seem to be more important. First of all, HCV is capable of escaping host Abs via rapid mutation. Anti-HCV are not protective and, in most cases, are a marker for disease. In contrast, an initial vigorous response by CD4+ T cells and memory CD8+ T cells is critical in preventing chronic infection. A weak initial response almost invariably leads to chronic infection.

Although a strong cellular immune response is necessary for the host to eradicate HCV, it can also cause liver damage due to CD8+ T cells killing infected hepatocytes. Thus, the goal of a vaccine is to induce a strong enough initial immune response to clear infection without causing severe acute hepatitis.

Both prophylactic and therapeutic vaccines are being developed using DNA plasmids, recombinant viruses
or bacteria, and VLPs. VLPs are an attractive approach because they have a particulate multivalent structure that is more immunogenic than soluble proteins. Research is focused on defining CTL epitopes and designing appropriate vaccine constructs. The desired immune response of the host includes the generation of both neutralizing Abs against HCV envelope proteins, E1 and E2, and T cell-mediated immunity against HCV proteins.

There are several challenges to the development of HCV vaccines. First, there is no suitable small animal model. Chimpanzees are effective as a model but are expensive and difficult to acquire. Also, until very recently, it has been difficult to grow large amounts of HCV in vitro. Finally, and most importantly, HCV has a high degree of genomic diversity. Its RNA genome readily mutates, especially in the envelope region. The many genotypes, subtypes, and quasispecies allow the virus to escape immunological control.

Despite these challenges, there has been much recent success in vaccine development. It was discovered that spontaneous eradication of the virus occurs in up to 50% of acute infections. This clearance is associated with specific immune responses to the virus. Infected humans and chimpanzees who mount an early, multi-specific CD4+ Th and CD8+ T cell response to HCV proteins can eradicate the virus by targeting multiple epitopes spanning basically all of the HCV antigens. In addition, activated T cells secrete proinflammatory cytokines such as interferon gamma, which is directly antiviral for HCV in cell culture and temporally associated with large reductions in viral load during acute infection.

Furthermore, due to recent capabilities in propagating HCV efficiently in cell culture, it has been found that patients infected with HCV cross-neutralize pseudoparticles derived from many different HCV genotypes: thus, there is evidence that a broad cross-neutralizing Ab to HCV may exist and could be used in vaccine strategies. Finally, studies involving the use of recombinant HCV envelope glycoproteins, gpE1 and gpE2, as vaccine antigens found that the highest responses resulted in animals being completely protected against infection. Even in lower-responding animals, most did not progress to the chronic condition.

Therapeutic vaccines are also finding success. Recent trials used an alum-adjuvanted recombinant gpE1 antigen that was able to boost humoral and cellular immune responses to gpE1. Therapeutic vaccines are especially important in view of the current treatments available, which use costly interferon alpha and ribivarin over extended periods of time (6-12 months). These treatments are associated with significant side-effects and result in sustained viral responses in only about 50% of patients.
Clearly, vaccination against HCV has the potential to control an important cause of global morbidity and mortality. An HCV vaccine will be reasonably cost-effective when used in the general population.

In conclusion, there are currently safe and effective vaccines being used for influenza virus; better vaccines will soon be available. In the near future, it is expected that vaccines will also be available for HPV and HCV. Widespread education is key to worldwide acceptance of vaccination as a preventative and therapeutic means to decreasing disease burden.

(The reference list for this answer has been lost somewhere in transmission.)

**Why Vaccinate**

Despite recent successes in reducing the incidence of infectious diseases, approximately 15 million of 57 million annual deaths worldwide are currently estimated to be directly attributable to infections. This burden is most evident in developing nations where coordinated vaccination programs are limited or non-existent. Global viral outbreaks will remain a significant threat to the human population unless effective vaccines are continuously developed and administered worldwide. Three viruses that impact humans throughout the world today are human papilloma virus type 16 (HPV 16), influenza virus, and hepatitis C virus. The development of effective vaccines against these viruses would undoubtedly save millions of lives per year around the world and reduce the public health and economic burdens associated with these infections. This review will focus on the risks posed by each of these viruses and provide information regarding recent progress made toward the development of vaccines. I will begin by discussing human papilloma virus 16.

Cervical cancer and precancerous cervical lesions pose a significant problem in women’s health. Approximately 470,000 cases of cervical cancer are diagnosed worldwide each year, and nearly half of these cases are fatal. Clinical, epidemiological, and molecular studies have all identified human papilloma viruses as the major causative agent in cervical cancer. In fact, 99 percent of all cervical cancer patients examined test positive for the presence of high-risk HPVs, which include types 16, 18, 31, and 45. Type 16
alone accounts for 50 to 60 percent of cervical cancers, and type 18 accounts for another 10 to 12 percent of all cases. Although screening in the United States has reduced the number of cervical cancer cases to around 10,000 per year, cervical cancer is the second leading cause of cancer-related death (behind breast cancer) for women in the developing world.

HPVs belong to a family of small double-stranded DNA viruses that exhibit strict species and tissue tropism. The virus infects immature basal keratinocytes, and different phases of permissive viral growth accompany distinct stages of keratinocyte differentiation and maturation. HPV is released from the cell upon natural apoptotic death, which occurs at a relatively high frequency for keratinocytes in the epidermis. Since cell death is normal, the inflammatory response is often absent, and the newly released viral particles can establish a chronic infection without recognition by the immune system. Most patients are able to respond to HPV infections with a strong CD8 T cell response along with the production of neutralizing antibodies. However, those individuals that do not mount an effective immune response to HPV are susceptible to chronic infection and possibly cancer.

Since the early 1990’s, both therapeutic and prophylactic vaccines against HPV 16 have been under consideration. Therapeutic vaccines would raise a CD8 T cell response, which would kill virally infected cells and clear the virus from affected individuals who have been identified through screening. However, prophylactic vaccines could be administered to individuals in early adolescence, before they become sexually active, so that HPV 16 infections are never established. Both Merck and GlaxoSmithKline are developing prophylactic vaccines against both HPV 16 and HPV 18. Both companies have based their vaccines on the L1 coat protein of the virus, and both vaccines are currently in phase III clinical trials.

A protective L1 protein has been difficult to synthesize since it must be expressed in a tertiary form and assembled as a multimer for neutralizing antibody to be generated. L1 coat proteins have been artificially synthesized either by inserting the L1 gene into plasmids for expression in yeast or by introducing the gene into recombinant baculoviruses for expression in insect cells. In these cells, the L1 coat proteins are exogenously expressed and self-assemble into L1 virus-like particles (VLPs). These empty virions appear morphologically identical to normal HPV and contain the major epitopes required for
a neutralizing antibody response. The dominant antibody produced \textit{in vivo} against HPV VLPs is of the IgG1 subclass.

The neutralizing antibodies generated by L1 VLPs appear to be type specific and little or no cross-reactivity exists between different HPV types, meaning that HPV 16 VLP immunization will only protect an individual from subsequent HPV 16 infections. GlaxoSmithKline’s vaccine is a bivalent HPV 16/18 vaccine, and Merck’s vaccine is a quadrivalent HPV 6/11/16/18 vaccine. Therefore, these vaccines have an extended range of protection. Initial results from trial studies have shown that both vaccines are safe and effective in immunizing uninfected women against high-risk HPV 16/18 types. Since cervical cancer is often fatal once established, vaccination against HPV may be the only way to reduce the frequency of this type of cancer in present and future generations. While human papilloma virus infection is usually only a threat to sexually active women, influenza viruses are a major threat to the entire human population.

Influenza has been estimated to infect 25-50 million people per year in the United States and cause between 30,000 and 40,000 deaths. If these numbers are extrapolated to the rest of the world, the global burden of influenza would be near 1 billion cases and 300,000 to 400,000 deaths per year. Influenza outbreaks are seasonal, and as with most other infectious diseases, the risk of severe influenza-associated complications is highest among children and the elderly. Three pandemics have occurred in the past century (1918, 1957, and 1968). The 1918 pandemic was the most severe of these outbreaks, and about 50 percent of the world’s population was infected. An estimated 20 to 50 million people were killed, and world population growth was depressed for 10 years following pandemic.

Cyclic influenza pandemics are driven by a phenomenon known as antigenic drift, whereby point mutations in the viral surface proteins hemagglutinin (HA) and neuraminidase (NA) leads to continuous emergence of new viral strains. Human influenza viruses can also acquire new genes from avian or other animal influenza viruses in a phenomenon known as antigenic shift. When this occurs, the virus can express an entirely new glycoprotein subtype. These alterations lead to the emergence of new viral strains against which there is little or no pre-existing immunity in the population.

Influenza vaccines are currently derived from inactivated virus grown in the allantoic cavity of embryonated chicken eggs. While these vaccines are effective in preventing influenza infections in vaccinated individuals, production is slow. Chicken eggs are less than ideal due to the low susceptibility of
summer eggs to infection and the possible presence of adventitious pathogens. The WHO estimates that more than 1.2 billion people worldwide today would be at risk in an emerging pandemic, and vaccines would have to be administered in a two-dose regimen. The absolute world vaccine production capacity using the chicken egg system is estimated to be only 900 million doses. Therefore, new, higher throughput production methods need to be developed in response to likely influenza pandemics.

Several pharmaceutical companies are currently developing mammalian cell lines including PER.C-6, MDCK, and African green monkey kidney (Vero) cells for influenza virus production. It is also now possible to develop a vaccine against any new strain of virus using reverse genetics, which allows for the production of influenza vaccines from cloned cDNA. In this process the virus is first attenuated by mutagenizing the HA1/HA2 cleavage site of the new strain. Then, the HA and NA genomic segments are transferred into a prototype influenza A strain, which can be grown on Vero cells in culture. The modified influenza A strain is then purified from the Vero cell culture and used for vaccination.

In addition to live attenuated influenza vaccines, subunit and DNA-based vaccines are also being developed. Naked DNA vaccines have proved to be poorly immunogenic in humans, but subunit vaccines hold more promise. One class of subunit vaccines are being developed using recombinant HA protein produced in insect cell cultures. These vaccines are now in phase III clinical trials. Another class of subunit vaccines has been created by genetically fusing the influenza virus transmembrane protein to the hepatitis B core antigen. The fusion protein spontaneously assembles into a virus-like particle that provides protection against the subsequent challenges with the influenza strain.

Rapid development and distribution of vaccines against potentially pandemic influenza strains will be essential to mitigate the economic impact an avian influenza could have. The cost of an avian flu pandemic has been estimated at about 167 billion dollars for all industrialized countries combined, and this figure only accounts for direct medical costs and lost worker productivity. The last virus that I will discuss is hepatitis C.

Hepatitis C was discovered in 1989 as an agent responsible for non-A, non-B hepatitis infection. HCV has since been recognized as a major cause of acute and chronic liver disease as well as hepatocellular carcinoma worldwide. The World Health Organization estimates that up to 170 million people are infected with HCV. The virus causes chronic infections in 75 to 85 percent of individuals
exposed. It is the most common bloodborne infection in the United States, and it is the leading cause of liver disease in developing nations. The prevalence of HCV is between 1 and 2 percent in most industrialized nations. However, the WHO estimates that approximately 6 percent of people in Pakistan are infected, and up to 22 percent of Egyptians have HCV infections. HCV is opportunistic in individuals infected with HIV, where approximately 25 percent of HIV patients have HCV co-infections.

HCV is a hepatotrophic, positive-strand RNA virus belonging to the Flaviviridae family of enveloped viruses. Before the development of diagnostic tests, HCV was most commonly transmitted through blood and organ transplantation. Today, HCV primarily infects intravenous drug users and their sexual partners. The problem is most significant in the prison population where 20 to 40 percent of inmates are infected. Studies of this virus have been held back by the lack of cell lines or small animal hosts that are capable of sustaining HCV replication. Until very recently, the only available model for studying HCV infections has been the chimpanzee.

Developing strategies for the creation of a vaccine against HCV has proven difficult due to considerable genetic heterogeneity of the virus, the fact that both humans and chimpanzees can be easily reinfected following re-exposure, and the ability of HCV to cause chronic, persistent infections. However, recent progress has been made, and we now know that spontaneous clearance of the virus occurs in up to 50 percent of infected individuals. Studies of acute HCV infections in chimpanzees suggest that the virus elicits a strong type-1 interferon response and vigorous polyclonal CD4+ and CD8+ T cell responses. However, the T cell response becomes weak and narrowly focused in individuals with chronic infections. The ability of HCV to evade innate immunity during chronic infections is thought to be due to expression of viral proteins that can block the phosphorylation and effector functions of host interferon. HCV can also suppress NK cell function by blocking interferon. With regards to evasion of adaptive immune responses, the HCV epitopes that both T cells and B cells recognize are hypervariable and subject to frequent mutation.

The current therapy for patients with chronic HCV infections is a combination of pegylated IFN-α and ribavirin. Pegylation is a process whereby polyethylene glycol (PEG) is attached to protein molecules in order to increase the protein’s half-life in vivo. Ribavirin is a nucleoside, guanine analogue. The drug works by inhibiting inosine monophosphate dehydrogenase (IMPDH), which is required for viral
replication. Unfortunately, the medication causes haemolytic anemia in many patients. Therefore, the development of a therapeutic vaccine could improve the quality of life for HCV-infected individuals.

A wide variety of vaccine approaches are currently under investigation. One of the most promising of these approaches involves the use of recombinant HCV envelope glycoproteins gpE1 and gpE2 as vaccine agents. These two proteins associate to form a non-disulfide linked heterodimer that is assumed to resemble the pre-virion envelope structure. Chimpanzees that responded with high antibody titers against gpE1/gpE2 were completely protected during subsequent HCV challenges.

Although lower responding animals did become infected, the majority of infections were acute and abortive rather than chronic and persistent. In order to determine whether the vaccine could protect against heterologous strains of virus, these researchers vaccinated nine chimpanzees with recombinant gpE1/gpE2 from different HCV strains and subsequently challenged all of the animals with the common HCV-H strain. They found that while none of the vaccinated animals were protected against acute infection, all but one chimpanzee completely cleared the virus, and no chronic infections were established.

The introduction of vaccines, antibiotics, and modern hygiene practices in the 20th century has contributed to significant declines in diseases that have plagued humans for millennia. In spite of this progress, we still face vaccine production shortages in the United States and Europe. Vaccine research, development, and production are often not high priorities for pharmaceutical companies because of low profit margins for many vaccines. Even though vaccines are often less lucrative than other, high-priced products, they do have economic value. The measles, mumps, and rubella vaccine (MMR-V) saves at least $16.34 in direct medical costs for each $1.00 spent, and the diphtheria, tetanus, and pertussis vaccine (DTP) saves at least $6.21 for each $1.00 spent. In addition, vaccines can prevent the incalculable human suffering that accompanies dangerous viral infections. Therefore, many scientists advocate government funding of vaccination programs.

Current research on human papilloma virus, influenza viruses, and hepatitis C virus show that progress is being made in understanding the infectious cycles of these viruses and developing strategies for vaccines against these agents. There are clear benefits to vaccination. A vaccine against HPV 16/18 would virtually end the threat of cervical cancer in women. The continuous development of vaccines against emerging and re-emerging strains of influenza will be needed to combat potential pandemics, and a vaccine
against hepatitis C would dramatically reduce the number of liver transplants required worldwide. Therefore, vaccines have, and will continue to improve the quality of life for people in all parts of the world.

Sources


