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# Understanding Pathogenicity: A Workshop for the BWC Meeting of Experts

**Workshop Summary**

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*Prepared under the auspices of the IAP Biosecurity Working Group*

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## CONVENING ORGANIZATION AND SPONSORS

The workshop and summary were produced under the auspices of the Biosecurity Working Group of IAP: The Global Network of Science Academies. IAP, formerly known as the InterAcademy Panel on International Issues, is a network of 107 of the world's academies of science. Its primary goal is to help member academies work together to advise citizens and public officials on the scientific aspects of critical global issues. The IAP Biosecurity Working Group was established in 2004 to undertake IAP's work at the intersection of biosciences and security. The Working Group now includes the academies of Australia, China, Cuba, Egypt, India, Nigeria, Pakistan, Poland (chair), Russia, United States, and United Kingdom and concentrates on two issues: a) education about dual-use issues in the context of responsible conduct of science, and b) implications of trends in science and technology (S&T) for the operation of the Biological Weapons Convention (BWC) and other nonproliferation treaties.

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## SUMMARY

A workshop was held in August 2014 under the auspices of the IAP Biosecurity Working Group to discuss understanding and modulating pathogen virulence mechanisms and host immune responses. The workshop focused on two complementary strategies for combating infectious diseases: targeting pathogen virulence factors and modifying a host's immune responses. These issues were directly relevant to the 2014 intersessional focus of the Biological Weapons Convention (BWC). An understanding of pathogenicity and immunology has the potential to be misapplied to create pathogens with increased virulence or to decrease the effectiveness of responses to infection. Alternatively, advances in this understanding offer promising new strategies in disease treatment. The workshop brought together approximately 35 scientists from academia and industry, scientific and technical experts from BWC delegations, and members of stakeholder communities interested in BWC issues. The workshop did not attempt to arrive at consensus conclusions, although several points were made by multiple participants, including the caution that novel approaches to alter host and pathogen responses are possible but enormously complex. The methods discussed present interesting opportunities, and would likely be used as additional lines of defense in concert with traditional therapeutics. The presentations at the workshop also raised the point that lines of research may have unexpected positive, as well as potential negative results for other fields of study. As a result, many participants highlighted the need for continuing communication between scientists and policy-makers and for members of the scientific community to be aware of how they present the findings and implications of their work. An open question of significant interest remains the issue of how to evaluate the risks and benefits of certain areas of research and the control of resulting information: who should determine whether the research is conducted, how the results are distributed, and based on what criteria?

## INTRODUCTION

Advances in science and technology can have profound implications for non-proliferation regimes such as the Biological Weapons Convention (BWC). As part of its annual program of intersessional meetings and five year review conferences, the BWC considers relevant scientific developments in order to ensure that the treaty keeps pace with a changing landscape and takes into account the impact of developments on treaty goals and implementation. In 2014, the scientific focus of the BWC is on "advances in the understanding of pathogenicity, virulence, toxicology, immunology and related issues." These issues are at the very heart of the BWC in that understanding pathogenicity might have the potential to be misapplied to create pathogens with increased virulence or to decrease the effectiveness of responses to infection, but advances may also offer promising new strategies in disease response.

A workshop preceding the 2014 Meeting of Experts organized under the auspices of IAP: The Global Network of Science Academies brought together approximately 35 scientists from academia and industry, scientific and technical experts from BWC delegations, and members of stakeholder communities interested in BWC issues. The workshop focused on two complementary strategies for combating infectious diseases: targeting pathogen virulence factors and modifying a host's immune responses. In addition to presentations, the meeting discussed potential applications and implications of this field of discovery for the BWC and the biosecurity community (see Appendix for the workshop agenda, discussion questions, and participant list). This report summarizes the presentations and discussions that occurred.

### Overview of host-pathogen interactions

*Kenneth Berns, University of Florida*

The first workshop speaker, Kenneth Berns, provided background on the immune response to pathogenic organisms. He began by pointing out that infectious diseases have been the major cause of human mortality throughout recorded history. As we have become more proficient in preventing and treating infections, life spans have increased, causing other major causes of death, such as cancer and cardiovascular disease, to become more common. Three advances in particular have been important in preventing and treating disease: the provision of clean water, immunization, and the development of antibiotics. Despite these successes, various infectious diseases, such as AIDS/HIV, tuberculosis, malaria, measles, influenza, dengue, Ebola and Chikungunya remain major threats to human health and a reminder that we live in a milieu in which microbes are ubiquitous. In fact, not only do we live in close proximity to multitudes of microbes, but they are an inherent component of the human body. Nevertheless, Berns suggested that we survive because we have developed sophisticated systems of defense in the form of the immune system.

The immune system is complex and has two parts: first, the innate immune system, which recognizes general molecular patterns and affords an immediate or short-term

response which can slow or inhibit infections. These early activities buy time for the second part of the immune system, the adaptive response, to become activated and trigger antibody production, allowing the cell mediated immune response to deal with extracellular and intracellular pathogens, respectively (Figure 1). However, just as humans have evolved sophisticated immune systems to deal with microbes, microbes too have adapted to elude human host defences. The microbes use strategies including changing surface proteins to avoid recognition, synthesising decoys to fool the immune system, or inactivating the protective pathways of the host. In some cases, this process is well understood; in other cases, the cause of damage at the molecular and/or cellular level has not been resolved.

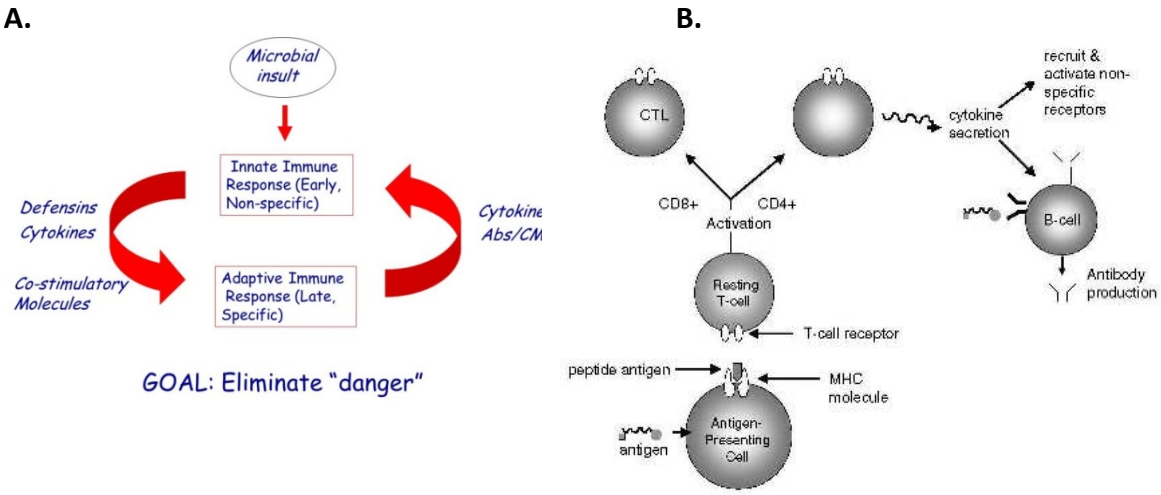


**FIGURE 1 Components of the immune system**. **A**. The innate and adaptive immune responses act in synergy to respond to microbial infection. **B.** The adaptive immune system has two arms—one that leads to the production of antibodies largely targeted against extracellular pathogens, and one that leads to the destruction cells that are infected with intracellular pathogens. SOURCE: A. Figure courtesy of Stefanie N. Vogel, University of Maryland School of Medicine. B. Institute of Medicine. 2001. *Multiple Sclerosis: Current Status and Strategies for the Future*. Washington, DC: National Academies Press.

Berns went on to suggest that vaccines represent one of the great success stories of medicine, although many pathogens remain for which we have not yet been successful in developing effective vaccines. The second great achievement in the war against infectious diseases has been the development of antibiotics and antivirals. With increasing knowledge of the molecular mechanisms underlying replication, it was possible to develop effective antivirals against such diseases as influenza, AIDS, and hepatitis C infection. The development of such drugs remains particularly important in the maintenance of public health defenses.

For all the benefits of advances in biology, it is also apparent that there has been a history of biology being misapplied for hostile purposes. Indeed, most biological research can be classified as dual-use. However, it is so-called “dual-use research of concern” (DURC) that is a particular worry, with seven types of experiments defined as DURC by the U.S. National Science Advisory Board for Biosecurity (NSABB). One classic

example of DURC is the work done in Australia on the mousepox virus and cytokine IL-4. This type of research raised two key questions:

1) To what extent, if any, should some types of experiments be prohibited or conducted under circumstances where dissemination of the results should be limited?
2) Should some information be redacted, and, if so, who should determine those who might have access?

Berns noted that there have been a number of recent cases when authors and journal editors mutually agreed to redact some details, but this may not always be the case. It would be preferable to deal with such questions early on, ideally prior to the commencement of research, an approach pursued in the United States, and something which could be considered at the international level. In the ensuing discussion, participants queried whether papers had been turned down because of potential biosecurity or biosafety concerns. Although the speaker was not aware of any papers which had been rejected outright on these grounds, he was aware of examples where editors worked with authors to come to voluntary agreements to redact and revise manuscripts prior to publication. Attempting to revise papers after the fact, however, remains problematic.

**Introduction to the workshop's focus**

*Nancy Connell, Rutgers New Jersey Medical School*

Following Kenneth Berns's introduction to the immune system and to potential scientific and policy concerns that may arise from dual use biological research, the workshop chair, Nancy Connell introduced the specific topics of the day's agenda.

Connell introduced the focus of the meeting by making reference to Article X of the BWC, which obligates states to avoid hampering economic and technical developments and encourages the fullest possible exchange and cooperation in biological sciences. She also drew attention to Article XII and the importance of reviewing science and technology as part of the established five year review conference process, particularly as the BWC was created in 1972, a time when scientists were just beginning to clone genes. Since then, there have been remarkable developments in the life sciences. To keep abreast of such developments, a new process emerged from the 7$^{th}$ Review Conference in 2011 to address specific topics during the 2012-2015 intersessional process. Developments in pathogenicity and their implications for the Convention was the topic under discussion at the 2014 Meeting of Experts and was the focus of this workshop.

The workshop's morning and afternoon sessions discussed areas of active research that rely on novel strategies to combat infectious microorganisms. Most traditional strategies to control diseases rely on boosting production of antibodies directed against a bacterial pathogen through vaccination, or on delivering drugs designed to kill a

pathogen by disrupting key components of its replication and growth cycle. However, selective pressure can rapidly lead to the emergence of antibiotic resistance, which has become a global problem. An alternative approach to controlling infection targets virulence mechanisms a pathogen uses to overcome host defence systems. Such virulence strategies can include production of bacterial toxins, secretion of factors that alter the host's immune reaction, formation of protective biofilms, and many others. Over the past few years, advances have continued to be made in understanding the complex interplay that occurs between host and pathogen following infection and interest has grown in exploring alternative control strategies. For example, by not directly killing the pathogen, anti-virulence approaches may be subject to less selective pressure to develop resistance and may help preserve the body's normal microbial flora.

## TARGETING PATHOGEN VIRULENCE FACTORS

*Fredrik Almqvist, Umeå University*

The first speaker of the session, Fredrik Almqvist, focused on "pilicides" and "curlicides" and the role of chemistry-based screening approaches to explore how molecules can be exploited to improve health. The speaker highlighted the significance of antibiotics and the importance of the discovery of penicillin. However, he suggested that there had been limited developments in the field of antibiotics since WWII and that antibiotic resistance was on the rise, with good bacteria (commensal strains) potentially spreading resistance and creating problems in the future.

Almqvist indicated that bacteria have a system of communication that enables them to exploit weakened immune systems and attach to the host, sometimes invading in order to replicate. The formation of biofilms of aggregates of microorganisms and secreted matrix molecules, which are assembled by multiple species, are also of significant concern as they can be capable of withstanding antibiotics. Furthermore, location of bacterial arrival in the body can be important. For example, *Escherichia coli* (*E. coli*) bacteria in the stomach are relatively unproblematic, but *E. coli* in the urinary tract may attach and replicate. The infection can be difficult to control, with the bacteria invading deep into host cells where they can lay dormant, causing recurrent infections later.

Almqvist pointed to the importance of understanding the biology behind such processes, specifically the role of adhesive fibers, or "pili," which enable some bacteria to attach to hosts cells. Building on such an understanding, Almqvist's team developed a class of pilicide compounds that can block the chaperone-usher molecular pathways used by the bacteria and inhibit assembly of the pili, thereby slowing down the process of bacterial infection. His team has screened numerous compounds for their ability to inhibit pilus formation, with the intention of making a battery of chemical variations to control the activity of pili (Figure 2). This strategy may have implications for dealing with disease. For example, the only current treatment available for tuberculosis is a cocktail of antibiotics, and the use of pilicides offers an alternative means of targeting bacterial virulence.

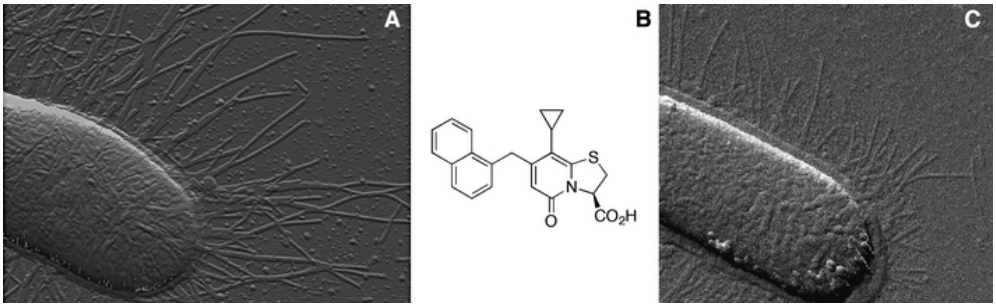


**FIGURE 2 Chemical pilicides affect expression of bacterial pili.** A. Bacterium expressing pili, B. an example of a chemical pilicide. C. Reduced expression of pili on a bacterium exposed to the pilicide. SOURCE: Reprinted by permission from Klinth, J.E. et al. Impairment of the biomechanical compliance of P pili: a novel means of inhibiting uropathogenic bacterial infections? *European Biophysics Journal* 41:285-295, copyright 2012.

*Abigail Male, University of Southampton*

The second presentation in the morning session focused on protein-protein interactions. Such interactions control most cellular processes, but they remain underexplored and targeting these interactions with conventional drug discovery methodologies remains a challenging, unexplored territory. Abigail Male stated that high-throughput screening has yielded some success, but that a general method for rapid screening of very large libraries of molecules would be of great value. She has approached this challenge using genetic selection, which has several advantages over traditional methods for drug discovery. In combination with a large molecular library, it can become a powerful method for uncovering inhibitors of protein-protein interactions. In Male's research, she has been able to screen around 100 million cyclic peptides for inhibitors of a chosen protein-protein interaction. Using this method, developing an active peptide sequence which can inhibit selected protein-protein inhibitors is feasible within the space of two months.

The bacterium *Bacillus anthracis*, the causative agent of anthrax, is able to infect both humans and animals and can be produced in vitro and used as a biological weapon. Male reviewed information on anthrax pathogenesis, specifically drawing attention to the binding of anthrax Protective Antigen (PA) protein to the human CMG2 receptor on cells (Figure 3). An inhibitor of this protein-protein interaction could disrupt the cell signalling cascade initiated by anthrax protein-cell receptor binding, potentially mitigating or preventing anthrax toxicity. To explore whether this presented a viable therapeutic option, Male and her team created a "reverse two-hybrid system" to study the interaction of CMG2 with PA with a view to screening via a SICLOPPS (Split-Intein Circular Ligation of Peptides and Proteins) library, which can be used for the synthesis of a large number of cyclic peptides. Male's team has so far screened four cyclic peptide libraries and identified several sequences of potential utility. They had further tested their compounds in vitro to explore whether they were able to prevent anthrax lethal factor from entering into cells, indicating they had managed to disrupt the protein interaction.

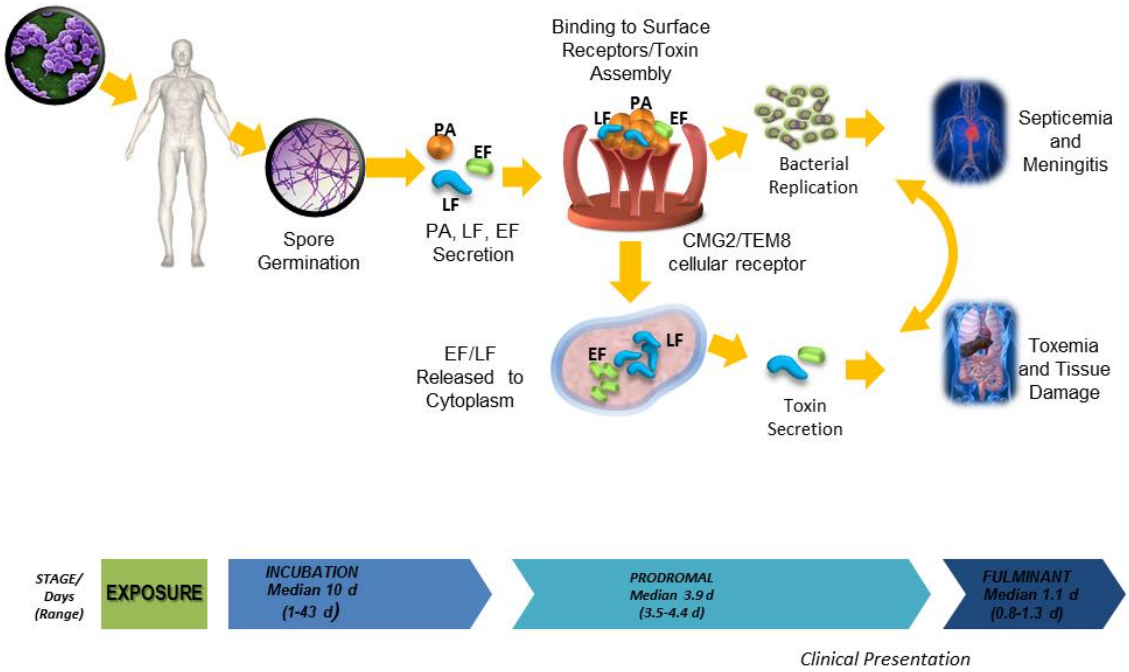


**FIGURE 3 Process of anthrax infection and disease progression.** The binding of anthrax toxin PA (protective antigen) to the cellular receptor CMG2/TEM8 is essential for the internalization of anthrax toxins LF (lethal factor) and EF (edema factor), which lead to many of the downstream symptoms of infection. Both Dr. Male and Dr. Posillico discussed strategies that block the interaction of anthrax PA with CMG2/TEM8. Dr. Male discussed the use of high throughput genetic screening techniques to identify protein molecules that disrupt the interaction. Dr. Posillico discussed the development of an antibody that binds to PA and prevents it from binding to the cellular receptor. SOURCE: Figure courtesy of Elusys Therapeutics, Inc.

Male concluded by noting that her team is working on second-generation inhibitors and had found molecules that were potentially more effective in interfering with the targeted protein-protein interaction. During the discussion, questions were raised about the normal function of CMG2, a transmembrane protein that is induced during capillary morphogenesis and which is hijacked by anthrax PA. Male indicated that the protein's normal function was not yet clear. She also indicated that the approach of disrupting such protein-protein interactions could have potential therapeutic application not just for infectious diseases, but also for non-infectious ones.

*Elizabeth G. Posillico, Elusys Therapeutics, Inc.*

The third speaker discussed an antibody-based approach to targeting bacterial virulence factors, focusing also on those expressed by *Bacillus anthracis*. Elizabeth G. Posillico indicated that this approach had much potential, but took time to come to fruition and meet the necessary safety requirements. She first reviewed the process of anthrax infection: after inhalation, spores are taken up by host phagocytic cells, such as alveolar macrophages and dendritic cells, and transported to lymph nodes where they germinate, secreting toxins. In its early stages, anthrax disease is asymptomatic and

early detection remains difficult – however, once anthrax becomes systemic, the toxins contribute to a shock-like syndrome, leading to death.

The mailing of letters containing anthrax spores that occurred in the United States in 2001 ("Amerithrax") demonstrated the limitations of available antibiotics, which were only able to help half of those who contracted inhalation anthrax as a result of exposure. The incident raised interest in finding alternative public health responses to anthrax. In response, Elusys Therapeutics explored the process of infection from pre-exposure to treatment through both intravenous and intramuscular routes. Posillico also drew attention to the importance of anthrax protective antigen (PA) because of its role in the conduit into the cell for anthrax toxins lethal factor (LF) and edema factor (EF), which subsequently interfere with immune defenses. She outlined how Elusys Therapeutics had been developing an antitoxin, ETI-204, designed to neutralize PA and prevent downstream toxin formation and immune cell apoptosis. Animal trials with the drug demonstrated that PA levels in untreated primates were higher than in ETI-204-treated primates, indicating that the drug shows promise in neutralizing the toxin quickly at low levels. The brain, spleen, liver, and lymph nodes of animals prophylaxed with ETI-204 were examined after 56 days with no bacteria detected.

In conclusion, Posillico stated that the ETI-204 antitoxin neutralizes the protective antigen of *B. anthracis* when administered during active infection. Moreover the anti-toxin limits dissemination of vegetative *B. anthracis* to blood and peripheral organs without undermining innate immune cell functions. In the discussion that followed, participants enquired as to the efficacy of using ETI-204 in conjunction with antibiotics, such as levofloxacin, ciprofloxacin and doxycycline; in response, it was suggested that this would be useful to consider further. Others asked about the circulating half-life of ETI-204, which is 21 days.

*Michael Wong, Sarepta Therapeutics*

In the final presentation of the morning session, Michael Wong began by discussing the effects of antibacterial agents on the broader microbiome, and the unintended ripple effects that can be generated by interventions. The research Wong presented focused on the use of a Phosphorodiamidate Morpholino Oligomer (PMO) platform to modify gene expression (Figure 4). The PMO platform is able to target mRNA in vivo and it offers the possibility of high specificity, good stability, and broad versatility. Wong indicated that PMO could have applications in the modulation of host responses to active infection, such as through modification of the expression and production of pro-inflammatory cytokines or pathways.

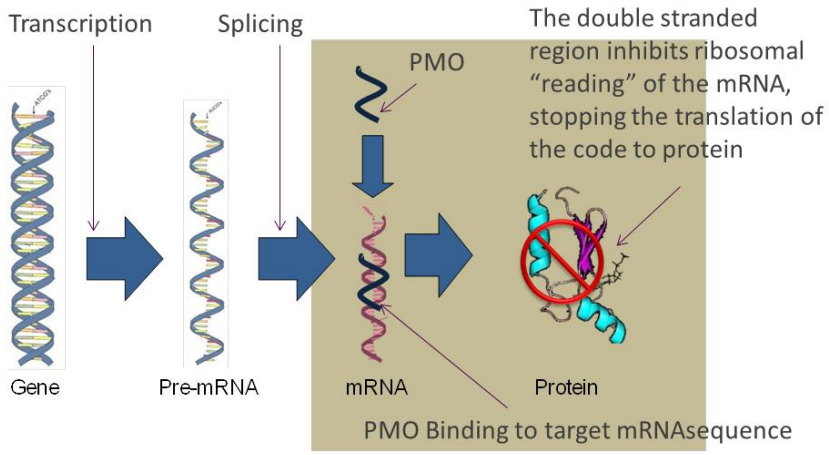


**FIGURE 4 Mechanism of action of PMO**. PMO alters gene expression by binding to RNA and blocking the cellular step in which an RNA molecule is translated into a protein. SOURCE: Figure courtesy of Sarepta Therapeutics (permission pending).

The PMO platform has been successfully tested in animal models in response to viral threats such as a West Nile virus outbreak in penguins (2002) and concerns over the emergence of pandemic influenza (2009) and dengue virus (2010), with efficacy for flu and dengue shown in mouse and ferret models. In the case of pandemic flu, for example, the Sarepta Therapeutics team responded to a U.S. Department of Defense (DOD) Transformational Medical Technology request for rapid response capabilities, and moved from concept to compound in seven days. Several Investigational New Drug (IND) submissions have been filed with the U.S. Food and Drug Administration (FDA). More recently, the platform has been tested in response to bacterial agents, such as a drug-resistant *Acinetobacter*.

The Sarepta team has also been involved in developing medical countermeasures against Ebola and Marburg viruses under the Food and Drug Administration's (FDA's) animal rule, for the purpose of protecting warfighters in areas where these viruses are endemic. These filoviruses have a 21-day incubation period and high mortality rates, and to date, no therapeutics are available although vaccines are being developed. The PMO platform is being used to target key viral genes understood to subvert host immune responses and combinations of different PMOs targeting different key genes can be used. Significant increases in survival rates in primates infected with Ebola or Marburg have been observed. Wong's team applied deep-sequencing techniques to samples of the virus recovered from infected animals to check for evidence of mutations in the binding site they had targeted with the PMO treatment, but none were found. Wong concluded that the PMO approach had been proven safe and effective in the short-term in animal models and in initial Phase I clinical trials in healthy human adults.

In the discussion, participants asked when Sarepta Therapeutics would be able to start further human trials. Wong indicated that interest in the PMO platform had been growing and that the DOD and the FDA had been helpful in streamlining the process

where possible. A further question was asked regarding the shelf life of the Sarepta Ebola virus therapeutics; Wong indicated that it was good for at least three years and currently appeared to be stable. Others asked about the practical implications of the technology and how the therapy would be utilized. Wong replied that the approach was originally designed for warfighters, not mass prophylaxis. However, it could be useful in post-exposure treatment of health workers and those that had come into contact with Ebola victims. It was still unclear whether it would be possible to achieve widespread treatment for Ebola with the current technology.

## DISCUSSION OF IMPLICATIONS AND RELEVANCE TO THE BWC FORUM

During the discussion session that followed the morning's presentations, participants drew attention to the convergence between biology and chemistry, which was reflected in many of the research areas discussed and is a topic of growing interest for both the BWC and the Chemical Weapons Convention. The importance of drawing out further implications of the research covered in the session for the BWC was raised. It was suggested that all participants should think beyond Article I and the potential for misuse or hostile applications of new research. The positive implications of developments in science and technology for the Convention should also be highlighted, such as the role developments could play in the provision of assistance in the event of a violation of the Convention.

Other participants drew attention to challenges in the timelines for new drug development and regulatory approval, as well as costs. Drug development frequently proves more complex than anticipated and participants queried whether more could be done on the supply side to facilitate the development of medical countermeasures. The lack of a commercial market for countermeasures to diseases such as anthrax was raised, in that companies may be entirely dependent on government funding in this area. This necessitates a significant commitment on the part of states, but also on the part of private companies, who are required to justify their selection of research areas to investors and shareholders. Still other participants raised the issue of patents, noting that exciting research in academia can be patented too early from an industry perspective, because of the pressure to publish. It was suggested that changes to the patent system or measures such as extending the lifetime of a patent can be helpful to stimulate some areas of infectious disease research.

## MODIFYING HOST IMMUNE RESPONSES

The afternoon session focused on modulating the immune responses that result from infection with a pathogen. As Kenneth Berns discussed at the start of the meeting, the host immune response is a complex interplay of cells and signaling molecules. A key question is whether these defense mechanisms can be transiently and safely modulated to overcome the virulence strategies of infectious agents.

*Diane Williamson, Defence Science and Technology Laboratory (DSTL)*
The first speaker in the afternoon session addressed the topic of manipulating the host immune response for therapeutic benefit. Diane Williamson began by reiterating how pathogens have evolved to evade aspects of host immunity and that difficulty and delays in diagnosis remain, with treatment sometimes required without a complete knowledge of the causative pathogen. Accordingly, there remains a need for therapies with a wide spectrum of action. One avenue for the development of novel solutions can be based on understanding pathogen and host interactions and developing means of identifying opportunities to manipulate host immune responses.

One way to build such an understanding is to compare RNAs expressed in the organs of infected and unexposed mice, using bioinformatics to aid in the interpretation of the data. This aids researchers in the identification of potential targets in the host and builds a better understanding of the disease process. Such an approach provides a wealth of output data on, for example, inflammatory response and granulocyte adhesion. Williamson illustrated the utility of this approach by describing the characterization of lung epithelium in mice infected with the bacterium *Francisella tularensis,* showing how this knowledge can be used to identify potential therapies that inhibit pathogenesis and protect the lung.

Williamson proceeded to outline how the human immune response can overcompensate when dealing with certain infections, resulting in a "cytokine storm," a process whereby too many immune cells become stimulated, potentially leading to sepsis, organ failure, and death (Figure 5). Intervening in this process through vaccines, anti-microbials, or anti-inflammatories to promote enhanced microbial clearance is important in restoring the balance and returning the host to a state of well-being. Anti-inflammatories are one particularly useful approach that researchers at DSTL have successfully used in conjunction with antibiotics to improve survival rates of infected mice. DSTL researchers have also investigated strategies for enhanced microbial clearance by activating dendritic cells (cells that stimulate an adaptive immune response) ex vivo and transferring these cells into mice infected with the bacterium *Burkholderia pseudomallei.* The animals treated with the activated dendritic cells showed significantly reduced bacterial loads.

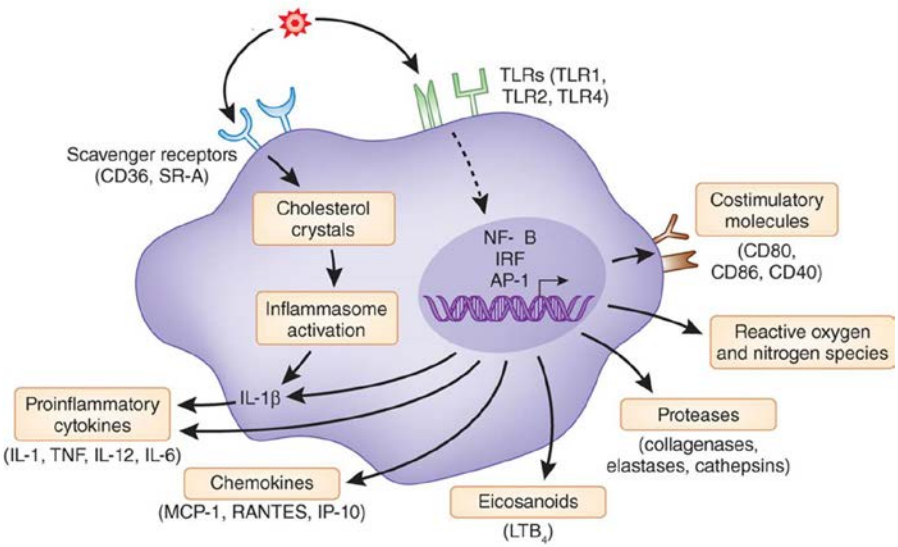


**FIGURE 5 Activation of the immune system**. Interactions with an antigen, such as a pathogen, lead to the production of immune stimulatory molecules. A harmful excessive reaction, or "cytokine storm," can sometimes result from this stimulation. SOURCE: Reprinted by permission from Macmillan Publishers Ltd: Hansson, G.K. and A. Hermansson. The immune system in atherosclerosis. *Nature Immunology* 12:204-212, copyright 2011.

Williamson concluded by stating that we need to understand host cell responses in order to manipulate such responses for benefit, either by blocking or activating host cell targets depending on the context. Accomplishing these manipulations is difficult because the host response is highly complex and interactive. Nevertheless, certain kinds of manipulation can demonstrate a significant impact on microbial clearance and survival.

In the discussion, participants asked whether the beneficial applications of this research could be reversed and used for hostile purposes. Williamson indicated that the complexity of the research made it difficult to reverse the direction of application for hostile purposes. Other participants queried whether it was possible to handle immune suppression early on, to which Williamson responded that it was difficult to stop and that intervening too early can have additional repercussions, which are difficult to anticipate and control.

*Alan Cross, University of Maryland*

The second speaker of the afternoon session began by reiterating that there is increasing antimicrobial resistance among clinical isolates of bacteria and that the antimicrobial pipeline has been drying up. These problems are compounded by the fact that new bacterial challenges are emerging, suggesting that alternative approaches to antimicrobial therapy must be considered. One such approach is through "non-antibiotics" that block pathogen virulence factors, inhibit triggers for biofilm induction and maintenance, or temporarily block host factors required for bacteria to replicate or

cause disease. One of the problems with targeting virulence is that these approaches are all largely pathogen-specific.

Cross accordingly presented two examples of potential host-directed therapies. One approach would be to stimulate host innate immunity in order to promote broad-based antimicrobial activity. This approach has the advantage of enabling early treatment before the causative agent is identified, but runs the risk of causing inappropriate activation of the immune system or tissue damage. A second host-directed strategy would be to inhibit a pathologic host response, essentially controlling the cytokine storm and facilitating a return to immunologic homeostasis. This approach points to the need for new classes of therapeutics that dampen inflammation while allowing pathogen elimination to continue, and underscores the importance of broad-spectrum, host-directed drugs that are effective against multiple pathogens.

Cross presented an example from the Kaempfer laboratory, which developed peptide antagonists that can be used to mimic inhibiters of pro-inflammatory cytokines, essentially manipulating the elements that trigger a host cytokine storm. Using animal models, the researchers demonstrated that intravenous delivery of an inhibitory peptide decreased cytokine storms and protected mice from a lethal challenge with the bacterium *Streptococcus pyogenes* in the absence of antibiotics, even when administered as late as five hours after infection. The researchers achieved similar success in protecting mice from a lethal challenge with *E. coli*. Cross concluded by stating that host-oriented therapy for infectious diseases has the potential to provide broad-based antimicrobial activity. Such a host-focused approach can enable early treatment of suspected infections before the causative organism is identified and potentially reduces the risk of selecting for antimicrobial resistance. However, inappropriate innate immune modulation could lead to tissue damage or immunosuppression and, accordingly, the work of identifying and understanding the mechanisms of suitable therapeutic agents is still in the early stages.

*Daniel Kalman, Emory University*

The final speaker focused on host-targeted chemotherapeutics for infectious diseases. Daniel Kalman described studies of how pathogens move into cells, survive within them, then exit, and the resulting immune response the cells launch against the pathogens. Researchers are using such knowledge to identify means to interfere with the process and to produce broad-spectrum anti-pathogen therapeutics. Kalman suggested that a starting point was the convergence of microbiology and oncology because pathogens utilize many of the same cellular and biochemical signalling pathways that are disregulated in cancer. More specifically, Kalman and his research team are addressing the question of whether pathogens utilize tyrosine kinases, similar to those expressed in the host, in pathways associated with motility and whether anticancer drugs that target these kinases could be employed to inhibit microbial pathogenesis. Several bacterial and viral pathogens have been investigated, including pathogenic *E. coli* (EPEC, EHEC);

poxvirus (vaccinia, variola); Filoviruses (Ebola, Marburg); *Mycobacterium tuberculosis* and *Franciscella tularensis*, among others.

Kalman noted that poxviruses and enteropathogenic *E. coli* (EPEC) bacteria have similarities in how the pathogen exploits the host's signaling systems for actin motility, which involve tyrosine kinase enzymes (Figure 6). Using the tyrosine kinase inhibitor drug Gleevec, which was originally designed for cancer treatment, researchers were able to limit poxvirus motility and reduce viral spread within mice, allowing them to survive an otherwise lethal dose. Kalman identified a number of other microbes that employ related tyrosine kinases, against which Gleevec was likewise effective. He also noted that Gleevec reduced intracellular survival of *Mycobacterium tuberculosis* in infected macrophages. Gleevec appeared to be useful even against antibiotic-resistant strains of tuberculosis, something that is being further explored along with studies co-administering Gleevec and antibiotic therapies.

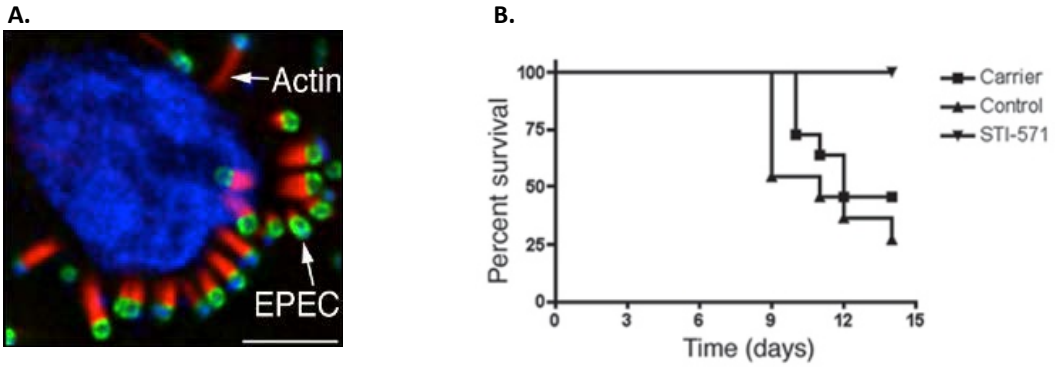


**FIGURE 6 A.** The pathogen EPEC can make use of host cellular pathways associated with actin motility. **B.** The drug STI-571 (also referred to as imatinib mesylate or Gleevec) can affect this actin pathway and represents a potential host-directed approach to reduce pathogen motility. For example, treatment with Gleevec increases survival in mice infected with vaccinia virus. SOURCE: A. Figure courtesy of Daniel Kalman, Emory University School of Medicine. B. Reprinted by permission from Macmillan Publishers Ltd: Reeves, P.M. et al. Disabling poxvirus pathogenesis by inhibition of Abl-family tyrosine kinases. *Nature Medicine* 11:731-739, copyright 2005.

Kalman indicated that dosing matters significantly to the effectiveness of the Gleevec therapy and is working to determine the optimum dose. The basis for this dosing effect appears to be stimulation of the production of myeloid cells in the bone marrow (which play roles in innate immunity) at a dose lower than that used to treat cancer. This effect mimics a normal response to infection. Once dosing in humans is better understood, Kalman's team plans to conduct human safety trials of the drug co-administered with antibiotics in patients with antibiotic-resistant tuberculosis. Kalman concluded by stating that host-directed therapeutics can be used to treat infections caused by an array of pathogens, because many pathogens use conserved biochemical pathways. Some of the pathways used by pathogens are disregulated in cancer, so potentially useful drugs may already be on hand. He also noted that lower dosages of drugs such as Gleevec, which stimulates a myelopoitic response, may be useful in treating infections, including those

arising from pathogens that don't utilize the specific pathways targeted (e.g., the bacterial genus *Franciscella*). Finally, he remarked that this work is rooted in observations about pathogen interactions with the host, illustrating the continued necessity of supporting basic science efforts.

In the discussion, participants asked about the response of Gleevec to granulocyte-colony stimulating factor (GCSF). Kalman indicated that the effect of GCSF is fairly limited in terms of the myeloid cell population and is virtually ineffective against infective diseases. He added that the drug works to induce all of myeloid cells and that his team had results showing that there is no discernible effect on the specific activities of the myeloid cells.

## DISCUSSION OF IMPLICATIONS AND RELEVANCE TO THE BWC FORUM AND IDENTIFICATION OF KEY MESSAGES

In the final session, participants again turned their attention to the implications of the developments discussed during the workshop and their relevance for the BWC. Participants discussed how the examples of modulating host responses could be exploited for hostile purposes, for example by manipulating the host's immune response or facilitating pathogenesis. It was suggested that this was effectively the thrust of the research on mousepox virus and Il-4, noted in the first talk of the day. As a result, the possibility of making potentially negative modulations to a host immune response exists, although other participants indicated that it can be difficult to switch the objectives and directions of complex research from peaceful to hostile purposes.

Participants also drew attention to the complexity of such research, highlighting the initial difficulties faced by those without significant expertise in understanding pathways and therapies discussed over the course of the workshop. In this regard, one message for the policy community that could support more informed discussion of the risks of dual-use research would be to highlight the complexity of many of the areas being studied. Similarly, other participants indicated that access to research data alone would not be enough to enable broad-scale misuse of developments, and that natural threats remained a greater concern. Additional participants highlighted the importance of stressing the positive benefits of such potentially dual-use research to policy actors in the BWC forum, and the role such research could play in, for instance, the provision of medical countermeasures assistance in the event of a violation of the convention.

Drawing on the recent discussions around influenza "gain-of-function" research, several participants raised the importance of independent risk-benefit analyses to inform how to move forward. Some participants at the workshop suggested that a consideration of the risks should be done at an early stage; however, other participants noted that claims over both benefits and risks remain contestable and uncertain, even after the fact, and would be even more ambiguous at the beginning of the research process. In this sense, a discussion of risks and benefits at an early stage, while likely preferable, would also be inherently more uncertain and problematic. This point was reinforced by the unforeseen

potential of some research to have unexpected positive results for other fields of study, like how oncology research had informed tests of existing anti-cancer drugs against pathogens utilizing similar biochemical pathways. Still other participants suggested a need for greater media acumen when presenting aspects of complex and potentially dual use science. It was suggested that some offhand remarks had been unhelpful in the discussions around gain-of-function research and had threatened to undermine efforts for a nuanced debate around such studies. Others drew attention to the diversity of H5N1 research, with different researchers taking different levels and types of precautions in their approaches.

Finally, the discussion raised the difficult issue of who should have responsibility for deciding on the risks and benefits of potential lines of research and, in cases where there are real concerns, who should determine how the results are distributed and based upon what criteria. Participants remarked that evaluations conducted by small groups or behind closed doors could be particularly problematic, as could unnecessary censorship or restriction of access to research.

## CONCLUDING REMARKS

The workshop brought together members of the scientific community and scientific and technical experts participating in the BWC forum to explore fields of research linked to the 2014 BWC intersessional program of work. In addition to exploring new research findings, the meeting discussed whether such fields have relevance to the implementation of the Convention. The workshop did not attempt to arrive at formal, consensus-based conclusions. However, several key points were raised by multiple participants over the course of the discussions and are provided here to help inform ongoing discussions:

- Novel approaches to targeting infection are theoretically possible but enormously complex: new methods will likely be used as additional lines of defense in concert with traditional therapeutics.
- There is the potential for research to have unexpected positive results for other fields of study, and vice versa, through convergence of biological fields of study.
- Greater media acumen in presenting aspects of dual-use science could help inform reasonable discussions and debates about the conduct of such research.
- Evaluation of risks and benefits and control of information remains an area of interest and debate: who should determine how research results are distributed? Upon what criteria should such decisions be made?
- Concern remains over mechanisms supporting continuing sustainability of advances, particularly in areas such as the development of anti-pathogen therapeutics where large commercial markets are not anticipated.
- The value of regular communication between scientists and policy-makers continues to be highlighted as a valuable part of nuanced discussions over the implications of scientific advances in fields that may have dual use potential.

## APPENDIX

### Agenda

*Chair: Nancy Connell, Rutgers New Jersey Medical School*

10:00 **Welcome and Introduction**

Overview of host-pathogen interactions
*Kenneth Berns, University of Florida*

Introduction to workshop focus
*Nancy Connell, Rutgers New Jersey Medical School*

10:45 **Targeting pathogen virulence factors**
*Fredrik Almqvist, Umeå University*
*Abigail Male, University of Southampton*
*Elizabeth G. Posillico, Elusys Therapeutics, Inc.*
*Michael Wong, Sarepta Therapeutics*

12:20 **Discussion of implications and relevance to the BWC forum**

12:45 **Lunch**

14:00 **Modifying host immune responses**
*Diane Williamson, Defence Science and Technology Laboratory (Dstl)*
*Alan Cross, University of Maryland*
*Daniel Kalman, Emory University (remotely)*

15:00 **Discussion of implications and relevance to the BWC forum**

15:30 **Identifying key messages and concluding remarks**
*Nancy Connell, Rutgers New Jersey Medical School*

16:00 **Adjourn**

### Discussion Questions

1. What are the most significant ways in which understanding and altering pathogen virulence mechanisms and host-pathogen interactions can contribute to improved disease treatments, defense against biological weapons, and other beneficial uses?
2. What key technical and policy barriers must be overcome to enable this field to advance effectively?
3. In what ways does research on understanding and altering pathogen virulence mechanisms and host-pathogen interactions raise potential dual-use concerns and what strategies might be useful in helping to mitigate potential concerns that arise?

4. When considering the risks and benefits of undertaking scientific investigations in this field and designing experiments, what key questions or issues do you think about?
5. What message would you most want to convey from the science community researching pathogenicity to the policy community concerned about the BWC and biosecurity issues (and in parallel, from the policy community to the scientific community)

## Participants

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University of Florida

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U.S. National Academy of Sciences

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Mission of Poland, Geneva

John Clements
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Nancy Connell
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Karen Fang
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Julie Fisher
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Meg Flanagan
U.S. Department of State

Elizabeth Frithz
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