HIV/AIDS: vaccines and alternate strategies for treatment and prevention

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The symposium “HIV/AIDS: Vaccines and Alternate Strategies for Treatment and Prevention” brought together HIV vaccine researchers to discuss the latest developments in the field. From basic discoveries in virus diversity and mechanisms of neutralization by antibodies to nonhuman primate research and clinical trials of vaccine candidates in volunteers, scientists are making great strides in understanding the mechanisms that may protect against HIV and pathways to achieve this protection through vaccination.

Keywords: HIV; vaccine; nonhuman primates; antibodies; clinical trials

Introduction

The Vaccine Science Discussion Group of the New York Academy of Sciences joined with the Global HIV Vaccine Enterprise to host a day-long symposium on May 19, 2010, titled “HIV/AIDS: Vaccines and Alternate Strategies for Treatment and Prevention.” The symposium was organized to mark HIV Vaccine Awareness Day, which falls annually on May 18. Most of the discussion focused on recent progress in HIV vaccine research.

Despite years of intensive research that has resulted in extensive scientific understanding of HIV biology, immunology, and epidemiology, a safe and effective HIV vaccine remains an elusive goal. Nevertheless, the recent RV144 trial in Thailand of a prime-boost vaccine candidate showed 30% efficacy—a modest but statistically significant result.\textsuperscript{1} The prime-boost strategy used a combination of two viral envelope proteins and a replication-incompetent canarypox vector expressing several HIV genes. Although the vaccine candidate prevented 30% of HIV infections in participating volunteers, as compared to placebo, it had no effect on plasma viral load in infected participants. These results drew increased attention to heterologous prime-boost approaches as means to stimulate both humoral and cellular immunity to elicit responses capable of blocking HIV acquisition. The renewed optimism in the field served as a backdrop for the conference.

Viral diversity

Michael Worobey (University of Arizona) gave a historical overview of the appearance, diversification, and worldwide spread of HIV. Discoveries of multiple simian immunodeficiency viruses (SIVs) in African nonhuman primates allow good understanding of the evolutionary history of these viruses.\textsuperscript{2} New findings of SIV-like viruses in mandrills of Bioko Island in the Republic of Equatorial Guinea date SIV ancestors to at least 100,000 years ago. The origins of the epidemic of HIV-1 are much more recent and trace back to the beginning of the 20th century and the subsequent spread of the virus from western Africa in 1969.\textsuperscript{3} Worobey's investigation of human samples from Kinshasa, Democratic Republic of the Congo, showed significant diversity of HIV in that town before the start of the worldwide epidemic.\textsuperscript{4} The subsequent spread of the virus is associated with multiple severe genetic bottlenecks. As a result, HIV-1 group M has diversified into multiple clades, which have different frequencies in different parts of the world. According to Worobey, these bottlenecks are associated with intrinsic low transmissibility of the virus, highlighting its vulnerability to prevention strategies. Recent data...
from other laboratories confirm his conclusions by showing the direct evidence for genetic bottleneck occurring during heterosexual transmission. Low per-contact transmissibility of HIV is promising for vaccine development because even a modest effect of vaccination on HIV infection will have a significant impact on the ability of the virus to spread in a population. While acknowledging the potential of vaccines, Worobey highlighted two challenges that continue to hamper the progress in HIV vaccine development: wide diversity of strains around the world and the seemingly unlimited ability of the virus to evolve while maintaining its replicative fitness.

Dan Barouch (Beth Israel Deaconess Medical Center) presented his approach to designing immunogens that specifically address the problem of high HIV diversity. Barouch and his collaborators from the Los Alamos National Laboratory have developed novel mosaic antigens and tested them in rhesus macaques. The immunogens are designed based on naturally occurring viral sequences using a sophisticated computational algorithm to optimize the coverage of known epitopes while preserving the integrity of the protein. Barouch used a mixture of two such immunogens and compared the immunogenicity to the more classical approaches of using consensus sequences or mixtures of genetically diverse viral strains. The mosaic approach showed both increased breadth (number of recognized epitopes) and depth (number of simultaneously induced responses by T cells) of immune responses elicited by this vaccine (Fig. 1). In addition, Barouch shared his research into the advantages of alternative adenovirus serotypes as vectors for delivering HIV genes as vaccine components. Preexisting immunity to the currently used adenovirus serotype 5 (Ad5) is believed to hamper the immunogenicity of this vector in people exposed to the wild-type Ad5 virus. Barouch’s laboratory investigated whether the Ad26 serotype would provide improved immunogenicity. Tested in human volunteers, the Ad26 vector was observed to be safe and immunogenic and was not suppressed by baseline cross-reactive Ad-specific T cell responses. Based on these results, Barouch concluded that Ad26 is a promising HIV-1 vaccine vector and should be further developed for clinical studies.

**Neutralizing antibodies**

Next, the discussion turned to the role of neutralizing antibodies and strategies to elicit them in vaccinated individuals. Sanjay Phogat (International AIDS Vaccine Initiative, IAVI) provided an overview of the recent progress in the field in isolating broadly reactive neutralizing antibodies (bNAbs). Isolating these antibodies and identifying their binding sites provides important information for rational immunogen design. The recent success

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Depth of CD4+ (top) and CD8+ (bottom) T lymphocyte responses after immunization with rAd26 vectors expressing mosaic, M consensus, clade B + clade C or optimal natural clade C antigens. Individual monkeys are depicted on the x-axis. One response variant or more than one response variants are shown for each epitopic region. Modified and reprinted by permission from Macmillan Publishers Ltd.
in isolating novel bNAbs was attributed to the systematic screening and selection of donor samples for potency and breadth of neutralization and serum-mapping studies. Isolation of bNAbs was further aided by innovations in high-throughput screening for antibody functions or binding to specific targets. Key targets of neutralizing antibodies include several regions of the viral envelope protein, such as the membrane-proximal external region and the CD4-binding site (CD4BS). However, the new bNAbs isolated by Phogat and his collaborators from the Scripps Research Institute bind to quaternary epitopes consisting of conserved regions in the variable loops on the surface of the Env glycoprotein. Thus, they provide new targets that vaccine researchers can use to design effective immunogens to elicit bNAbs. The identification of new antibodies targeting CD4BS by investigators at the NIH Vaccine Research Center (VRC) and at the Collaboration for AIDS Vaccine Discovery (CAVD) has reinvigorated the design of immunogens to present CD4BS to B cells. The VRC antibodies, like antibodies directed to the quaternary neutralizing epitope in the V2 and V3 regions of gp120, are very potent and have broad specificity, while the CAVD HJ16 antibodies are as potent and broad as the previously known b12 antibody. Current research looks to present these epitopes to the immune system in a way that focuses immune responses on these epitopes (Fig. 2). This requires creating proteins that form and present the necessary epitope without the context of the natural envelope trimer, whose properties divert the immune system away from neutralizing epitopes.

Susan Zolla-Pazner (New York University School of Medicine) described bioinformatics analysis of the HIV-1 envelope glycoprotein gp120 that revealed structural conservation in the second and third variable loops (V2 and V3) of gp120. Immunoc chemical, virologic, and bioinformatics data provided converging evidence of antigenic conservation in V2 and V3. Furthermore, immunological and functional studies of monoclonal antibodies that target quaternary neutralizing epitopes composed of regions of V2 and V3 provided evidence of cross-reactivity. These lines of evidence suggest structural and antigenic conservation for this new target that lies in the V2 and V3 regions of gp120. Zolla-Pazner supported the view that antibodies to these regions may mediate potent neutralization of a broad range of viruses and, therefore, that these regions of the HIV-1 envelope should be used as one of the targets of an AIDS vaccine intended to induce neutralizing antibodies. Zolla-Pazner highlighted the need for 3D visualization of this target either by physical methods (crystallography and NMR) and/or through molecular modeling. Using these approaches, Zolla-Pazner and her colleagues determined a generic structure for the V3 loop and applied it to the design of V3-scaffold immunogens that contain variants of this generic V3 structure. As one approach to increase immunogenicity, a chimeric protein was created that incorporated a V3 loop immunogen into the cholera toxin B. Her laboratory has now synthesized several such V3-scaffold immunogens and tested them for antigenicity and for immunogenicity in rabbits. They were shown to induce cross-clade neutralizing antibodies for several Tier 1 and Tier 2 viruses. This work constitutes a complete “cycle” of the reverse vaccinology approach to vaccine design and serves as a proof-of-principle: identify neutralizing monoclonal antibodies, characterize the epitopes they recognize, model these epitopes onto scaffolds, produce epitope-scaffold immunogens, immunize rabbits and elicit antibodies with activities similar to...
those of the original monoclonal antibody. Thus, the rational immunogen design approach for HIV vaccines is feasible and can be applied to existing and new targets.

**Nonhuman primate research**

Louis Picker (Oregon Health Sciences University) provided updates on his studies of the role of effector memory T cells (T\textsubscript{EM}) elicited using rhesus cytomegalovirus (CMV) vectors, in containing SIV infection. Because T\textsubscript{EM} cells reside close to the site of viral entry, these cells can potentially “intercept” infection within the first hours and days following infection when the virus is most vulnerable to immune control. Previous work from Picker’s laboratory showed that rhesus CMV vectors carrying SIV genes can elicit potent, long-lasting CD4\textsuperscript{+} and CD8\textsuperscript{+} T cell responses.\textsuperscript{16} These responses completely protected 50% of vaccinated macaques from progressive SIV infection after limiting dose intrarectal challenge with the highly pathogenic SIV-mac239 virus. This protection is distinct from that of previous T cell vaccines against SIV in its abruptness and extent. Protected macaques manifest a viral burst in plasma of varying size upon initial infection followed by immediate control of virus to undetectable levels. To date, this stringent control has been stable for more than 45 weeks in 94% of initially protected macaques. Interestingly, CMV vector-mediated protection is “all or none” in that the SIV-specific T\textsubscript{EM} responses elicited by these vectors do not provide any protection once the infection has become systemic. Taken together, these data indicate a novel pattern of protection consistent with

![Figure 3. Viral RNA/ microgram tissue RNA for various tissues from animals after vaccination with SHIV and then infection with SIV; also shown are titers of viral RNA in animals after CD8\textsuperscript{+} T cell depletion.](image-url)
very early $T_{EM}$-mediated control, likely occurring at the site of viral entry or early sites of viral replication and amplification.

Another approach to HIV vaccine development was presented by Chris Miller (University of California, Davis). Miller’s team vaccinated macaques with a live-attenuated chimeric SIV that carried the envelope of HIV (Fig. 3). Following vaginal SIV challenge several months after vaccination, there was complete protection (no PBMC SIV env DNA or plasma vRNA) in 16 of 43 (37%) immunized animals and protection from uncontrolled viral replication in 11 (26%) immunized monkeys that became infected with SIV. Thus, 27 of 43 (60%) of immunized animals were protected from SIV infection and/or SIV disease (CD4$^+$ T cell loss). This protection was associated with the presence SIV-specific CD8$^+$ T cells in the vagina but not the cervix on the day of SIV challenge. To determine whether this control was dependent on CD8$^+$ T cells, the researchers used a monoclonal antibody to deplete these cells, as well as natural killer cells, in five animals (Fig. 4). Depletion resulted in the loss of viral control. Of particular interest, animals that were immunized and then depleted of CD8$^+$ T cells had higher viral loads than the immunized animals. One possible explanation for this finding is the presence of the vaccine-elicited, SIV-specific CD4$^+$ T cells in the vaginal mucosa at the time of SIV challenge, which would support virus replication.

Both of these studies show the importance of T cells in viral control and highlight the need for continued investigation of CD4$^+$ and CD8$^+$ T cells using different viral vector platforms.

**Candidate vaccines**

Results of the RV144 trial in Thailand of an HIV vaccine candidate released in October 2009 showed a 31.2% reduction in HIV acquisition (Fig. 5).1 Jerome Kim (Military HIV Research Program) updated the audience on the progress in the post-hoc analyses of the trial. Laboratory studies show that even though more than 95% of volunteers who received the vaccine developed binding antibody against vaccine antigens, the level of antibodies quickly decreased more than 10-fold by 24 months after the last vaccine dose. Antibody-directed, cell-mediated cytotoxicity assays revealed lower levels of positivity than were observed in the earlier Phase II trial of the same candidate. The vaccine induced more CD4$^+$ T cell responses than CD8$^+$ T cell responses. Analysis of breakthrough infections indicated that vaccine and placebo recipients had distinct patterns of epitope recognition, suggesting a vaccine-mediated sieve effect—something that has been observed for the candidate tested in the Step trial. On the other hand, analysis of breakthrough viruses showed no differences in subtype distribution between HIV strains observed in the population during the screening period and in the incident infections during the trial. However, the sieve effect might be limited to specific sites in the viral genome, a possibility that can be tested once the single-genome analysis of breakthrough infections is completed. To follow-up on these results, MHRP...
is considering a number of trials that would test utility of different vaccine vectors and extend the findings to other risk populations (Fig. 6).

The post-hoc analysis also continues for the Step trial, which was stopped in 2007 because of the lack of efficacy observed during the midpoint analysis of the data.18 Juliana McElrath (Fred Hutchinson Cancer Research Center) reviewed the trial, which aimed to elicit the CD8+ T cell immune response with adenovirus vector Ad5 expressing gag, pol, and nef of HIV-1. Vaccinated volunteers showed detectable HIV-specific responses (mostly by CD8+ T cells), but of very low level.19 The vaccine did not lower HIV infection rates or postinfection plasma viremia. More importantly, the breadth of elicited responses was insufficient to recognize and respond to incoming viral strains (see Viral Diversity section above). The vaccine did prevent infection by viruses whose proteins were similar to those in the vaccine, but other strains were able to establish infection unimpeded.20

Harriet Robinson (GeoVax) and her colleagues have been exploring the modified vaccinia Ankara virus (MVA) as a potential vector for an HIV vaccine.21 The GeoVax DNA and MVA-vectored HIV vaccines express noninfectious, virus-like particles bearing native forms of Env (Fig. 7). A Phase I clinical trial (HVTN 065) testing prime-boost strategies has shown safety and unique patterns of immunogenicity. The full-dose regimen (using two DNA primes and two MVA boosts) elicited the highest levels of CD4+ and CD8+ T cell responses, whereas MVA priming and boosting (using three MVA inoculations) induced the highest response rates and titers of antienvelope antibody. Both regimens are currently in a Phase IIa study (HVTN 205). Similar regimens were recently tested in rhesus macaques using SIV239 prototypes of the HIV vaccines. The NHP model allows controlled virus challenge of vaccinated monkeys to test vaccine efficacy. Robinson used a high-dose repeat intrarectal heterologous challenge with E660 SIV (a dose of 40 to 400 times higher than experienced in a typical human exposure). The DNA/MVA as well as the much simpler MVA-only regimen provided comparable protection with 25% (2/8) of the challenged animals in both groups being protected against infection through 12 weekly challenges. Expression of granulocyte-macrophage colony stimulating factor (GM-CSF) in the DNA prime vector increased protection against the 12 weekly challenges.

Figure 5. Results of the RV144 trial in Thailand of an HIV vaccine candidate released in October 2009 showed a 31.2% reduction in HIV acquisition.

Figure 6. Trials that would test utility of different vaccine vectors and extend the findings to other risk populations.
to 70% (5/7). The search for correlates of protection showed that the GM-CSF-adjuvanted group had higher avidity anti-Env Ab, higher frequencies of neutralizing Ab for a tier 1 variant of E660 (E660.11) and higher frequencies of anti-HIV IgA in rectal secretions than the unadjuvanted DNA/MVA regimen. The use of the GM-CSF adjuvant for the GeoVax DNA/MVA vaccine regimen is being advanced into clinical trials as part of the GeoVax vaccine pipeline.

These reports emphasize the unique role of clinical trials in HIV vaccine research. In the absence of clear correlates of protection, clinical trials of vaccine candidates provide the unprecedented opportunity to investigate human immune responses to vaccines and the effectiveness of such responses in protection against HIV.

**Future paths**

The Global HIV Vaccine Enterprise (the Enterprise) is a worldwide alliance of organizations that have voluntarily agreed to work together to accelerate the development of a safe and effective HIV vaccine. The Enterprise recently embarked on a major update of its Scientific Strategic Plan, which will identify the priorities for the field for the next several years. Alan Bernstein, executive director of the Global HIV Vaccine Enterprise, presented the findings of the five Working Groups that were assembled by the Enterprise to evaluate the current state of HIV vaccine research, to identify key challenges and opportunities, and to make recommendations on how best to address them. Widespread agreement exists on the need to advance the current approaches to clinical testing of the efficacy of HIV vaccine candidates. Clinical trials have to be done faster, better, and more often and take advantage of the latest discoveries in fundamental science, of the most robust assays and technologies, and of innovative trial designs. Clinical trial capacity in countries where trials are conducted needs to be developed to allow more sophisticated sample collection and analysis. To optimize the use of available resources, the field needs to explore ways to facilitate open sharing of protocols, data, and latest findings. Bernstein also highlighted the importance of training a new generation of investigators, engaging industry in vaccine research, and attracting new sources of funding. These and other issues will be fully discussed in the Global HIV Vaccine Enterprise Scientific Strategic Plan, which will be published in 2010.

**Appendix**

**Conference organizers**
Sarah Schlesinger (The Rockefeller University), Yegor Voronin (Global HIV Vaccine Enterprise), and Jennifer Henry (New York Academy of Sciences); presented by Global HIV Vaccine Enterprise and the Vaccine Science Discussion Group.

**Conference agenda**
Wednesday, May 19, 2010
9:00 am  Welcome
   Jennifer Henry, New York Academy of Sciences
9:05 am  *Overview of the Challenges*
   Yegor Voronin, Global HIV Vaccine Enterprise
9:15 am  *Viral genetic diversity and the control of HIV/AIDS: challenges and opportunities*
   Michael Worobey, University of Arizona
9:45 am  *Novel Vectors and Antigens for a Next Generation HIV-1 Vaccine*
   Dan H. Barouch, Division of Vaccine Research, Beth Israel Deaconess Medical Center
10:20 am
Coffee Break
10:45 am
**Recent Progress in Isolating HIV-1 Broad Neutralizing Antibodies**
Sanjay Phogat, International AIDS Vaccine Initiative

11:15 am
**Using Epitopes Recognized by Monoclonal Antibodies as Vaccine Templates**
Susan Zolla-Pazner, NYU School of Medicine and Veterans Affairs Medical Center

11:50 am
**New Insights into Immunologic Vulnerabilities of Highly Pathogenic SIV**
Louis J. Picker, Oregon Health & Science University

12:30 pm
**A Protective Live-Attenuated AIDS Vaccine Suppresses Innate Immunity and Inflammation in Immunized Rhesus Macaques**
Chris Miller, University of California

1:10 pm
Lunch Break

2:00 pm
**Insights from Recent Clinical HIV Vaccine Trials That Can Guide Future Vaccine Designs**
Juliana M. McElrath, Fred Hutchinson Cancer Research Center

2:40 pm
**Clinical and Preclinical Studies for DNA and Recombinant MVA Vaccines Expressing HIV-1 Virus-Like Particles**
Harriet L. Robinson, GeoVax Inc.

3:15 pm
**Update of the Thai Phase III HIV Vaccine Trial: the Way Forward**
Jerome Kim, Walter Reed Army Institute of Research

3:55 pm
Coffee Break

4:20 pm
**HIV Vaccine Research Today, the Global HIV Vaccine Enterprise, and the Enterprise Scientific Strategic Plan**
Alan Bernstein, Global HIV Vaccine Enterprise

4:50 pm
**Panel Discussion: Where Are We Going and What’s Next?**
Moderated by Sarah Schlesinger and Alan Bernstein

Dan H. Barouch, Chris Miller, Mitch Warren, Louis J. Picker

5:25 pm
Closing Remarks
Sarah Schlesinger, The Rockefeller University

5:30 pm
Networking Reception

References


2009 H1N1 swine flu: the 2010 perspective

Doris Bucher,1 Terrence Tumpey,2 Anice Lowen,3 James Gill,4 Michael Shaw,5 James Matthews,6 Jose Galarza,7 Jennifer Minieri Arroyo,1 and Philip Ralph Dormitzer8

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In May 2009, as the H1N1 swine flu outbreak was in the early stages, a conference was held at the New York Academy of Sciences to discuss what was known about the virus and what was being done to stop the outbreak. In May 2010, a follow-up conference was again held at the New York Academy of Sciences, but now to discuss the H1N1 outbreak retrospectively. The report presented here summarizes the 2010 conference proceedings.

Keywords: swine flu; outbreak; H1N1; cross-protection; vaccine; pandemic; 1918 influenza

Introduction

A previous human swine flu conference held at the New York Academy of Sciences on May 28, 2009, occurred as the pandemic was developing, thus offering a “real-time” perspective from the presenters, including Michael Shaw (Influenza Division of the Centers for Disease Control (CDC)) and Scott Harper (New York City Department of Health and Mental Hygiene), who generously gave their time while dealing with the demands of monitoring the outbreak at the city, national, and international levels. At nearly the same time, on May 21, 2009, Doris Bucher (associate professor at New York Medical College) and colleagues in her laboratory had just completed production of the key “seed” reassortant NYMC X-179A, which provided high growth in eggs required for producing an H1N1 vaccine; the Influenza Division of the CDC quickly performed a complete evaluation of X-179A, declaring it to be an acceptable candidate for preparation of the vaccine.1

At the 2010 conference [see Appendix for the conference agenda] Doris Bucher provided an introduction to the current view of the 2009 H1N1 outbreak and its evolution to the present; she also introduced some of the key points of discussion at the 2010 conference:

- transmission characteristics of 2009 H1N1, which facilitated rapid spread of the virus
- pathology of the virus, as seen in fatal cases in New York City
- the current state of the pandemic and evolution of the virus
- challenges of 2009 H1N1 vaccine production
- several new approaches to preparation of influenza vaccines

Bucher provided a brief primer on influenza viruses to provide a context for 2009 H1N1, emphasizing the key role of the hemagglutinin (HA) antigen in developing influenza vaccines. The goal of immunization with the influenza vaccine is to generate antibodies to HA, because HA-specific antibodies neutralize the virus and provide protection against infection by circulating influenza virus. In contrast, the neuraminidase (NA) antigen of influenza is the target for the NA-inhibitor antivirals oseltamivir and zanamivir, which played a vital role in managing 2009 H1N1 infections, preventing morbidity and mortality. A major clinical difference between these two targets is that NA-inhibitor antivirals could be used for patient treatment as soon as 2009 H1N1 was identified; however, the HA-specific vaccine was not available until after the peak incidence of H1N1 swine flu in October 2009.
Bucher reminded the audience that this was the third encounter in the past hundred years with an influenza virus bearing the swine H1 (HA subtype 1) and N1 (NA subtype 1) surface antigens. The first encounter was the H1N1 virus responsible for the 1918 pandemic of swine flu; the second encounter was the appearance of swine flu H1N1 at Fort Dix in 1976, which, while not an epidemic or pandemic flu outbreak, led to the 1976 swine flu vaccine campaign that ultimately ended with significant adverse events; and the third encounter was the pandemic of H1N1 in 2009. In fact, 2009 H1N1 continues to circulate worldwide and cause disease. Consequently, the seasonal influenza vaccine formulation for the Northern Hemisphere in 2010–2011 will be trivalent, containing virus vaccine strains for two type A viruses, 2009 H1N1 and H3N2, and one type B virus.

Because adverse effects associated with the swine flu campaign of 1976 included a neurologic disease called Guillain-Barré syndrome (GBS), occurring at the rate of approximately 1/100,000 vaccinees, exhaustive testing was done to ensure the safety of the 2009 H1N1 vaccine.² It is estimated that the incidence of GBS with the seasonal vaccine is very low; reported estimates vary from no risk to about 1/1,000,000 recipients; in comparison, the 1976 swine flu vaccine had a 10-fold increased risk of GBS. Bucher reported that at a meeting of the strategic advisory group of experts at a World Health Organization (WHO) meeting in April 2010, no unexpected safety concerns were identified for any of the pandemic (H1N1) 2009 vaccines following administration of 350 million doses.³

Bucher also remarked that the scientific and governmental responses to the H1N1 pandemic were rapid and well coordinated, with groups from the CDC, the National Institutes of Health (NIH), and industry working tirelessly with maximal effort and intensity to ensure the H1N1 vaccine supply. Yet, while the 2009 H1N1 virus was transmitted much faster than vaccine was produced, tested, and distributed, the relatively broad availability of effective antiviral medications helped more to ameliorate the intensity of disease.

Transmission of H1N1

The ferret model

Terrence M. Tumpey (Centers for Disease Control and Prevention) presented a talk entitled “Transmission and Pathogenesis of H1N1 Influenza Viruses in Ferrets.” The 2009 (H1N1) influenza pandemic has resulted in laboratory-confirmed cases in over 210 countries with greater than 18,000 deaths worldwide (http://www.who.int/en), with a spectrum of human disease ranging from mild to severe illness and death. The pathogenesis of the virus in humans remains not fully understood; in particular, the ability of 2009 H1N1 virus to target cells in the lower respiratory and gastrointestinal tract is enigmatic. Moreover, it is not clear how the swine-origin H1N1 influenza virus strain acquired the ability to transmit efficiently in humans.

Tumpey discussed how the ferret has been used to model influenza infection since it was first isolated in the 1930s. Ferrets are naturally susceptible to influenza viruses and exhibit many clinical signs following infection (such as sneezing, fever, and nasal discharge) that are comparable to influenza-like illness in humans. Tumpey presented data from experiments with ferrets comparing the pathogenesis and transmissibility of three 2009 H1N1 virus isolates to a representative seasonal H1N1 virus, A/Brisbane/59/2007. In these respiratory droplet transmission experiments, three animals were inoculated intranasally with 10⁶ plaque-forming units of virus and inoculated-contact animal pairs were established by placing a naive (i.e., uninfected) ferret in each of three adjacent cages with perforated side walls, allowing exchange of respiratory droplets without direct contact. Direct-contact transmission experiments were performed similarly, except naive ferrets were placed in the same cage as each of the inoculated ferrets, where they shared a common food and water source. Transmission was assessed by titration of infectious virus in nasal washes and detection of virus-specific antibodies in convalescent sera. Three additional inoculated ferrets from each virus-infected group were used for assessment of virologic parameters.

Use of the ferret model revealed that in contrast to seasonal influenza H1N1 virus, 2009 H1N1 viruses cause increased morbidity and lung pathology, and replicate to higher titers in lung and intestinal tissues. With respect to transmissibility in ferrets, the 2009 H1N1 viruses spread efficiently by direct contact, comparable to seasonal H1N1 viruses, but with reduced efficiency by the respiratory droplet route compared with seasonal influenza viruses. The lack of efficient respiratory droplet transmission of the 2009 H1N1 viruses in the ferret model suggests that
additional virus adaptation in mammals may be required to reach the high-transmissible phenotype observed with seasonal H1N1 or H3N2 isolates in humans. Indeed, adaptation of the HA receptor-binding site (RBS) and polymerase basic protein 2 (PB2) appear to be important for pathogenesis and transmission of H1N1 viruses. The RBS of the 2009 H1N1 virus is typical of many other classical swine H1N1 viruses recently isolated in North America, and the necessary adaptive mutations in the HA surface protein required for efficient respiratory droplet transmission in ferrets is currently under study. With respect to PB2 protein, a single amino acid substitution from glutamic acid to lysine at position 627 supports efficient influenza virus replication at the lower temperature (33°C) found in the mammalian airway, and contributes to efficient transmission in ferrets. Tumpey concluded that PB2 position 627 mutation, which can appear through mutant selection or reassortment, along with changes in the RBS, should be closely monitored as markers for enhanced virus transmission.

The guinea pig model

Anice Lowen (assistant professor of microbiology at Mount Sinai School of Medicine, New York), in a talk entitled “Transmission of the H1N1 Pandemic Influenza Virus,” spoke about the transmissibility of the 2009 pandemic strain and focused in particular on data generated from the guinea pig model of influenza virus transmission. Lowen highlighted the fact that epidemiological measures of viral spread, including reproduction number \( R_0 \), generation time, and influenza-like illness attack rates calculated during the early months of the pandemic, suggest that the swine-origin H1N1 strain transmits among humans with comparable or slightly greater efficiency than does seasonal influenza virus. She then introduced the guinea pig as a model host and demonstrated that, as in humans, pandemic H1N1 strains transmit very well in guinea pigs and with similar efficiency, as do seasonal H3N2 influenza viruses. Specifically, Lowen and colleagues found that the A/California/04/2009 (H1N1) pandemic strain spread to four of four exposed guinea pigs when infected and naive animals were housed in the same cage (i.e., 100% transmission by the contact route) and that this same strain, as well as the A/Netherlands/602/2009 (H1N1) virus, transmitted with 100% efficiency by the aerosol route. The high transmission rate observed with the pandemic H1N1 viruses starkly contrasts with that observed for swine influenza virus isolates in the guinea pig model. Lowen presented results obtained with two North American swine viruses, sw/Texas/4199/1998 (H3N2) and sw/Kansas/7778/2007 (H1N1); both were found to transmit by an aerosol route to 25% of exposed guinea pigs. Thus, despite the fact that the genome of the pandemic 2009 H1N1 virus comprises gene segments that have been in circulation in swine for at least 10 years, the 2009 H1N1 virus transmits with much greater efficiency than swine viruses in both guinea pig and human hosts.

Lowen also presented results of experiments designed to assess the potential of preexisting immunity to seasonal influenza viruses to limit the spread of the pandemic strain. Guinea pigs were initially infected with a seasonal H1N1 or H3N2 subtype influenza virus and then, 3 weeks later, challenged with the pandemic H1N1 virus A/California/04/2009. For the challenge, each animal was either inoculated intranasally and then placed in the same cage with a naive guinea pig or simply exposed to an acutely infected guinea pig. In this way, the impact of preexisting immunity on transmission from and to previously infected animals was assessed. The results indicated that prior exposure to seasonal strains did indeed limit the spread of the pandemic virus, though the protection observed was not subtype specific. The reduction in transmission may therefore be due either to non-specific effects on the host that persist over a 3-week period or to an adaptive immune response that targets a component of the virus conserved among the seasonal and pandemic strains examined. These results, as well as human data collected during previous pandemics, suggest that the circulation of seasonal H1N1 and H3N2 influenza viruses in humans prior to the spring of 2009 most likely impacted the epidemiology of the pandemic.

The emergence of the swine-origin influenza strain during the spring in the Northern Hemisphere was also important in shaping its epidemiology; although transmission continued throughout the summer, peak influenza activity occurred only later, during the autumn and winter months. In addition, as Lowen pointed out, the timing of the epidemic peak in 2009 was very unusual: while most influenza outbreaks peak in January, February, or March, the pandemic strain showed its
highest activity in mid-October. These epidemiological observations raise an interesting question: Can the unusual timing of swine-origin H1N1 influenza virus spread be accounted for by differences in sensitivity to environmental conditions between this virus and previous human strains? Lowen demonstrated that, in the guinea pig model, aerosol transmission of a 2009 pandemic strain had a very similar dependence on humidity and temperature as a seasonal H3N2 influenza virus. Lowen’s suggestion, based on these data, is that the unusual seasonality of the 2009 H1N1 virus seen to date is most likely due to a relative lack of immunity against this virus in the human population, and her prediction, based on that fact, is that in the coming years novel H1N1 epidemics will adopt winter seasonality.

Pathology findings of fatal 2009 H1N1 infections

“Medicine, to produce health, has to examine disease.” Plutarch

James Gill (The New York City Office of Chief Medical Examiner (NYC OCME)) gave a talk entitled “Pathology Findings of Fatal 2009 Influenza A/H1N1 Infections in New York City.” The NYC OCME investigates unexpected, suspicious, and unnatural deaths that occur in New York City; its public health role includes surveillance and investigation of deaths due to possible communicable infectious agents. Gill mentioned that during the 2009 H1N1 outbreak, the NYC OCME investigated suspected influenza deaths, including all unexplained deaths due to febrile respiratory illness. Between May and July 2009, of 42 confirmed H1N1 deaths, 32 underwent autopsy; the findings plus those for two additional deaths seen in consultation were reported in the Archives of Pathology and Laboratory Medicine.

In this study, autopsy results and clinical information were reviewed, and airway swabs were analyzed by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) for H1N1 viral RNA. In addition, microscopic and microbiologic evaluations were performed, and tissue was examined by the CDC and the NIH for localization of H1N1 viral antigen by immunohistochemistry (IHC).

The majority of the 34 decedents (62%) were between 25 and 49 years of age. Frequent symptoms included fever (94%), cough (91%), or shortness of breath (73%). Comorbidities included obesity (72%), chronic heart and lung diseases, diabetes mellitus, and immunosuppressed states; a majority of the decedents (91%) had one or more comorbidities. Tracheitis, bronchiolitis, and diffuse alveolar damage (DAD) were noted in most decedents; influenza viral antigen was detected by IHC most commonly in the epithelium of the tracheobronchial tree, but in some instances viral antigen was also detected in alveolar epithelial cells, submucosal glands, and macrophages. Secondary bacterial pneumonia was detected in 55% of decedents, with Streptococcus pneumoniae as the most common pathogen. Thirteen decedents, pronounced dead on arrival at the hospital or at home, died from natural progression of H1N1 infection without medical treatment; of these, six had both bronchopneumonia and DAD, two had bronchopneumonia, and two had DAD.

Gill went on to describe how determination of the cause of death with proper death certification is crucial for accurate vital statistics. A cause of death is composed of three parts: the mechanism, immediate cause, and proximate cause. The proximate cause (also know as the underlying cause of death), the only component required on the death certificate, is defined as “that which in a natural and continuous sequence, unbroken by any efficient intervening cause, produces the fatality and without which death would not have occurred.” Moreover, the proximate cause of death must be an etiologically specific disease (or injury). In contrast, some physicians and lay coroners focus on the immediate cause or the mechanism of death and neglect to include the proximate cause of death on the death certificate. One common death certification error, for example, is to list bronchopneumonia as the cause of death. In fact, bronchopneumonia typically is an immediate cause, rather than the underlying cause, of death. In the vast majority of instances, bronchopneumonia is not a proximate cause of death because it is not an etiologically specific disease (i.e., many diseases, such as AIDS, emphysema, and lung cancer, may result in a bronchopneumonia). For a 2009 H1N1 influenza fatality, the death certification would commonly read: respiratory failure (mechanism) due to bronchopneumonia (immediate cause) due to novel influenza A (H1N1) viral respiratory infection (proximate cause); any contributing conditions (i.e., co-morbidities, such as obesity or hypertensive cardiovascular disease) would be listed in part two of the death certificate.
Gill ended by discussing the results reported in the *Archives of Pathology and Laboratory Medicine* study, which showed that most 2009 H1N1 influenza infections were self-limited and that deaths occurred in a younger age group (median age 37 years) than expected for seasonal influenza (over 65 years); immunohistological lung findings in fatal 2009 H1N1 influenza infection were similar to those identified in past pandemics; and bacterial coinfections of the respiratory tract were common. Preexisting obesity, cardiorespiratory disease, and other comorbidities were detected in the vast majority of decedents.

**CDC pandemic response**

Michael Shaw's (Centers for Disease Control and Prevention) talk entitled “CDC Pandemic Response: Testing Preparedness” began by recounting that in late winter/early spring of 2009, news reports appeared describing outbreaks in Mexico of respiratory disease of unknown etiology; but it was not until the causative agent had spread to the United States that this respiratory infection was identified as a novel influenza strain. This novel strain of the H1N1 subtype proved capable of rapid spread from person to person and established itself worldwide in a matter of weeks, displacing the previously circulating influenza strains causing outbreaks in months normally considered to be “out of season” for influenza. Shaw mentioned that while we may never know precisely how the novel H1N1 strain arose—with its previously unseen mixture of swine, avian, and human influenza virus genes—retrospective analyses of specimens from the early Mexican outbreaks support the idea that the virus first appeared in Mexico in a form capable of sustained transmission in the human population.

Since the first infections of humans with highly pathogenic avian influenza virus in Hong Kong in 1997, planning for pandemic infection had assumed that a new pandemic strain would most likely appear first in Southeast Asia, thus giving some advance warning to activate emergency operations plans in the United States. These plans included port of entry monitoring, containment and prophylaxis in initial outbreak foci, deployment of new diagnostic tests, and treatment guidance, and assumed the United States would have something of a “head start” on vaccine production and deployment before infections reached epidemic proportions.

According to Shaw, however, with a new pandemic strain of influenza virus (2009 H1N1) first recognized within U.S. borders, pandemic planning efforts of the federal and state governments were put to the test under unanticipated circumstances. By the time it was recognized as a novel influenza strain against which most people had no immunity, the 2009 H1N1 pandemic strain had already established itself in multiple locations in the United States, thus requiring a change in focus toward emphasizing mitigation and prevention rather than containment. State and local laboratories that were already operating at less than full capacity, due to budgetary constraints, were severely strained by the rapidly increasing workload.

Shaw said that the rapid detection of “unsubtypable” type A influenza viruses in California in April 2009 was made possible by extensive pandemic preplanning at the CDC, Health and Human Services (HHS) sister agencies, and state and territorial public health partners. The CDC Influenza Division already had in place a Food and Drug Administration (FDA) 510K-cleared seasonal assay, which had been deployed through the Laboratory Response Network to include all state public health labs. Joint CDC/Association of Public Health Laboratories training of personnel in 2008 and 2009, combined with funding to support service contracts for the required PCR diagnostic platform, meant the capacity was in place to receive the new diagnostic kit approved under the Emergency Use Authorization less than 2 weeks after the new 2009 H1N1 strain was identified and characterized. Diagnostic test reagents were rapidly disseminated to all state public health laboratories and virtually every national public health laboratory in the world.

Shaw continued by saying that the decision was made early to rapidly and freely share information on the 2009 H1N1 virus as it developed, including genomic sequence data, diagnostic and analytic protocols, and drug susceptibility. Virus isolates were also shared freely, ensuring that researchers worldwide would have access for research and development purposes. It soon became apparent that the virus was not changing substantially as it spread,
probably due to a lack of immune pressure in the affected populations. This simplified vaccine strain selection and allowed expert committees at WHO and FDA to recommend the A/California/7/2009 virus for vaccine production. As mentioned by Doris Bucher, a high-yield reassortant strain, NYMC X-179A, was produced at New York Medical College, Valhalla, and made available to manufacturers on May 23, less than 6 weeks after the pandemic strain was first identified.

The new pandemic H1N1 strain continued to spread from state to state as spring and summer progressed, causing outbreaks in schools and summer camps. A waning of activity toward the end of summer was short-lived and, as schools restarted in the fall, the epidemic quickly gathered momentum, finally peaking in mid-October in the United States. Vaccine production schedules for the domestically licensed egg-based formulations did not allow widespread distribution until the epidemic was already subsiding in the United States. However, the virus did remain almost entirely susceptible to antiviral therapeutic agents, such as oseltamivir, which was distributed to the states from the Strategic National Stockpile, and resistance to oseltamivir never exceeded 1% of the viruses tested during the first year. The vast majority of oseltamivir-resistant viruses were from patients on long-term therapy due to complicating conditions, and these strains remained sensitive to zanamivir, an alternative NA-inhibitor agent.

According to Shaw, this first pandemic of the 21st century was notable for successes, such as the rapid detection of the new strain and open sharing of information and laboratory reagents. However, it also highlighted deficiencies, especially in the rapid production, testing, and distribution of vaccines using existing systems. It is now also apparent that more effort needs to be applied to the development of rapid diagnostic tests that can be used in clinical settings that lack sophisticated molecular analytic equipment. Shaw concluded by emphasizing that the 2009 H1N1 pandemic resembled the “milder” 1957 H2N2 pandemic more than it did the devastating 1918 H1N1 pandemic in severity and mortality, which was fortunate for the United States and other populations. Clearly, however, lessons must be learned from the pandemic in order to better inform future planning and research required to prepare ourselves for the potential of an even more serious infection in the future. The events of the past year have shown that a new pandemic could occur at any time and in any country.

An industry perspective

James Matthews (Sanofi Pasteur) next gave a talk entitled “The 2009 H1N1 Pandemic—An Industry Perspective.” Since the first serious outbreak of the H5N1 virus in Hong Kong in 1997, many of those involved with pandemic planning initiatives had developed assumptions about the likely origin and severity of the next pandemic based on H5N1 and the previous three epidemics (1918, 1957, and 1968) of the 20th century. Based on the endemicity of H5N1 in bird populations throughout Southeast Asia, it was assumed that the next pandemic would originate from birds in a southeast Asian country and, after a short period of local and regional spread, it would become a global infection with high mortality. Matthews said that it was somewhat surprising, therefore, that the 2009 H1N1 pandemic originated in swine and was first detected in North America. Other aspects of the 2009 H1N1 also defied the planning assumptions; for example, in contrast to H5N1, the 2009 H1N1 pandemic had a low case fatality ratio and the elderly population was disproportionately spared. In the United States, there is a point estimate of ∼12,000 deaths, which is considerably lower than the 36,000 typically detected during seasonal influenza. Globally, as of June 2010, perhaps, a little over 18,000 deaths have been associated with the 2009 H1N1 pandemic.

Matthews noted that there was little heterogeneity in the H1N1 virus population even after a year of circulation, in marked contrast to the clades and subclades that characterize H5N1 viruses. As a consequence, the original A/California strains were well-matched to over 99% of the circulating strains and did not need to be “updated” during the pandemic. In addition, in contrast to the poor immunogenicity of H5N1 vaccines, the inactivated vaccine for H1N1 was highly immunogenic in all segments of the population, without the need for adjuvant. These results not only were contrary to those of the generally poorly immunogenic H5N1 vaccines but also were in stark contrast to the long-held assumption that any standard (i.e., unadjuvanted, conventional 15 μg dose) vaccine developed against a novel
influenza virus would likely require two doses in a naive population.

Matthews emphasized that, in general, the response to the 2009 pandemic was remarkable because without compromising the production and regulatory process a 2009 H1N1 vaccine that had been tested in all major segments of the population was available approximately four months after the WHO declaration of a pandemic. In fact, owing to the “mild” nature of the 2009 pandemic and the need for only a single dose of vaccine, some of the planning predictions were impacted. For example, except for relatively low vaccine availability in October 2009, throughout most of the immunization campaign there was far more vaccine available than was required. And after a short interval of demand in the third and fourth quarters of 2009 the U.S. population was generally not receptive to the immunization campaign. Although HHS had ordered approximately 230 million doses of vaccine to be produced, only between 81 and 91 million doses were administered to approximately 72–81 million people. Immunization rates outside of the United States were even lower on a population basis. In fact, by February 2010, manufacturers were instructed to discontinue shipments of finished vaccine to the CDC distribution centers. Current information indicates that fewer people received the 2009 H1N1 vaccine than those who normally receive the trivalent vaccine during the annual, seasonal immunization campaigns. Those who did receive the 2009 H1N1 monovalent vaccine appeared to be primarily individuals who also received the seasonal vaccine. Importantly, because of the “mild” nature of the pandemic there was no recommendation from the WHO to discontinue seasonal influenza vaccine production, so that seasonal and pandemic vaccine production continued in parallel, competing for manufacturing resources.

Matthews concluded his talk saying that we are fortunate that the 2009 H1N1 pandemic was “mild” and that the vaccine performed well, in terms of safety and immunogenicity. Clearly, the 2009 pandemic has provided us with the opportunity to reexamine some of our planning assumptions and implementation plans. There is some consensus that the WHO pandemic phases need to be reevaluated to include dimensions relating to severity and rates of transmission that better match the global response to the perceived threat. In order to shorten the period when vaccine is first available, said Matthews, we need to validate alternate methods of vaccine potency; equally important, we need to continue to expand global production capacity. To do this, continued efforts for greater acceptance of seasonal immunization must be made. Matthews continued by saying that it is only through increased, consistent uptake of seasonal vaccine that we can rationalize capacity expansion, and that we also need to improve our communications relating to the benefits of vaccination. As was seen in the United States and globally, there is little sense in urgently preparing millions of doses of vaccine if it has low public acceptance (i.e., use). Finally, as with other naturally occurring phenomena that have high probability of occurrence but no fixed interval, the 2009 H1N1 pandemic demonstrated that it is not possible to plan for a single type of event. Rather, a plan needs to be in place for a spectrum of severity and for focusing resources on the underlying fundamental processes of the health care system: surveillance, rapid diagnosis, stockpiles, capacity, and distribution channels. These measures not only will better prepare us for the next pandemic, but also will provide benefits for dealing with other public health emergencies as well.

**Influenza virus–like particles as vaccines**

Next, Jose Galarza (TechnoVax, Inc.) presented a talk entitled “Influenza Virus–Like Particles (VLP) as Effective and Economical Human and Animal Vaccines.” The unexpected emergence of the 2009 H1N1 pandemic influenza virus that swiftly spread around the world has again challenged our ability to rapidly and efficiently develop an effective vaccine. In contrast, the current method of flu vaccine development relies on embryonated chicken eggs and requires many months for manufacturing, making the response to this kind of rapidly spreading emergency inadequate. New technologies for creating and manufacturing a vaccine in a rapid manner, using a controllable and scalable system, are highly desirable. Significant progress has been made in this area. One of the most promising technologies for influenza vaccine development is based on virus-like particles (VLPs), structures that resemble wild-type influenza virus in size, morphology, and biochemical composition but are not infectious or able to replicate because they do not contain viral genetic material. VLP-based vaccines are produced in cell
culture systems using standard fermentation methods and purified from the culture supernatant; and because the VLPs are not infectious, vaccine inactivation (e.g., by treatment with heat or denaturing agents) is not required, which better maintains the antigenic properties of the VLP surface antigens.

Preclinical studies with VLP vaccines have demonstrated that they are highly immunogenic and efficacious in protecting small animal models against virus challenge. VLP vaccines have shown equivalent effectiveness when administered via either intranasal or intramuscular routes, allowing for alternative immunization protocols. In contrast to seasonal influenza vaccines that are formulated by blending three antigenically distinct virus strains (two type A viruses and one type B), VLP technology allows for incorporation of two antigenically distinct HA molecules on the surface of the same VLP; this approach permits the creation of bivalent VLPs that can be combined to formulate a tetravalent seasonal influenza vaccine (one bivalent with H1/H3 and one with two antigenically distinct type B HAs). This vaccine-development strategy reduces manufacturing steps and costs while increasing vaccine coverage. VLP technology thus enables production of noninfectious, multivalent, highly protective influenza vaccines from controlled cell culture systems and rapid vaccine development.

**Panel discussion**

*Philip Dormitzer, moderator (Novartis Vaccines and Diagnostics), Doris Bucher, Terrence Tumpey, Anice Lowen, James Gill, Michael Shaw, James Matthews, and Jose Galarza*

The panel discussion touched on major themes of the meeting, including the role of antigenic cross-reactivity in shaping the age distribution of illness; differences between seasonal and pandemic influenza; the public health impact of the vaccine; the speed of the vaccine response; prospects for using new technologies to improve future responses; and the use of animal models to understand pandemic influenza transmission and protection.

Dormitzer asked Tumpey about the implications of a paper that Tumpey had coauthored. The experiments described in the paper demonstrate the remarkable antigenic similarity between the 1918 pandemic influenza strain and the 2009 H1N1 pandemic strain. Presumably, the 1976 swine flu strain also resembled the 1918 and 2009 strains. During the H1N1 2009 pandemic, there was a relatively low disease burden among the elderly, who may have been infected by 1918-like strains and immunized with the 1976 vaccine. Dormitzer noted that “the 1976 vaccine campaign has been described as a 'fiasco'.” He asked Tumpey if, in contrast, the 1976 vaccination campaign might have contributed to low rates of illness among the elderly during the 2009 pandemic, making it “one of the greatest flu vaccines of all times and a paradigm of how we can prepare for the next pandemic.”

Tumpey responded that as soon as the 2009 H1N1 pandemic virus emerged in April he and colleagues examined the sequences and noticed that the antigenic site of the 2009 virus was identical to the 1918 virus and some older H1 strains. He said they found that animals immunized with 1918 virus showed good cross-protection against the 2009 H1N1 virus; he and others also obtained data (unpublished) showing that animals vaccinated with the 1976 virus-based vaccine were protected against the 2009 H1N1 virus. This long-term antigenic conservation reflects the natural history of influenza in the past century in which the 1918 virus “jumped” into pigs, in which there was probably little selective pressure, and the resulting H1 swine flu strains that emerged stayed antigenically similar through the 2009 pandemic.

Galarza said that his group has immunized animals with VLPs specific for 1918 virus and demonstrated significant cross-protection against challenge with a 1930 swine influenza virus, which circulated 12 years after the 1918 virus. On the basis of this, Galarza did not find it surprising that there is some cross-protection between viruses separated by a number of years of independent evolution.

Dormitzer asked Lowen to comment on the cross-protection against a pandemic H1N1 strain observed in the guinea pig model after infection with an antigenically dissimilar seasonal H1N1 or even an H3N2 strain. Lowen noted that the 1976 vaccine was given over 30 years ago and that it is not clear how long the protection would last from such a killed vaccine. Lowen said her study in the guinea pig model examined the effect of an actual infection (not immunization with a killed vaccine) on an influenza challenge given just 3 weeks later. And while she did not investigate the mechanism of protection, she suspects that the protective effect observed was
antibody mediated because they observed full neutralizing protection. Lowen said that she surmised that the cell-mediated response would be too slow to produce such protection, and that her group may study the mechanism of cross-protection in future experiments.

Dormitzer noted that Gill had found the pathology in the 2009 fatal H1N1 influenza cases to be similar to the pathology in fatal cases from previous influenza pandemics. Dormitzer asked Gill how similar the pathology of the two infections was to that seen in fatal seasonal influenza cases. Gill responded that “as medical examiners we don’t see a whole lot of seasonal influenza ourselves,” which creates a biased information base. He said, “a 70 year old with heart problems who dies of seasonal influenza is not a case that is reportable to us.” Gill also noted that comorbidities are likely to play more of a role in the elderly than in younger people. However, in the case of 2009 H1N1, the elderly were probably less likely to die from pandemic infections because of some immunologic protection against the virus.

Dormitzer asked Shaw about the impact of the H1N1 pandemic vaccines to date and in the future. Dormitzer noted that the vaccine only became available after the peak of disease and that, although 61 million Americans were infected with the pandemic strain, 180 million were not infected. Shaw responded that “it is going to be a while until we can take a stab at that...The true answer to your question is I don’t have an answer.” However, he ventured that the vaccine probably did not have a large effect on the past season, but that if the H1N1 pandemic virus strain returns next season the vaccine may provide some residual immunity or even priming effects. Shaw concluded that “it would have been better for everyone if the vaccine could have come out earlier.”

Dormitzer asked Matthews, “If you could have anything, what would you change to speed up the response so we can get the vaccine out earlier, next time before the peak?”

Matthews responded by saying that possible ways to accelerate the response include an alternative method for creating reassortants and a codified way of getting partially tested potential vaccine seed virus strains to manufacturers. Matthews proposed using high pressure liquid chromatography as a more rapid alternative vaccine release test. He also noted that having sufficient manufacturing capacity is an issue and that “more sitting capacity would help.” Matthews concluded by saying, “if we had had a clearer indication that there was a pandemic and stopped seasonal vaccine production, that would have helped.”

Dormitzer noted that an advantage of the old influenza vaccine technologies is that they are “tried and tested.” He asked Galarza whether a new technology, such as producing influenza vaccine antigens in Chinese hamster ovary cell lines, could be utilized successfully in the few weeks available from the time that a strain is selected to the start of manufacturing vaccine for a new flu season.

Galarza responded, “the strategy is to have a cell line with the core elements already set up and then come [in] later with genes that will be updated.” He continued that “alternatively, one could bank cells expressing a diversity of HAs from which to select.” He noted that “we are developing technologies to rapidly introduce HA and NA into cells that already express M1 and M2.” He referred to recent literature on broadly neutralizing antibodies generated in the laboratory using samples from human subjects, concluding that “we need to find the antigen that is inducing these antibodies. Then we can have a more broadly neutralizing vaccine.”

A listener submitted the following question over the Web: “Given the oversupply of 2009 H1N1 vaccine...will those leftover vaccines be administered?”

Matthews responded that, according to FDA regulations, expired vaccine may not be administered and that each manufacturer determines its own vaccine’s shelf-life: “Once there is seasonal vaccine on the market, the leftover vaccine from a previous season is not to be used.”

An audience member noted the concern for zoonotic influenza outbreaks (when a virus jumps from animals to humans) and asked: “[whether] the tremendous heterogeneity that we see in H5N1 is due to the multiple reservoir species of birds in Asia and Siberia...[rather than] the very narrow species diversity in livestock in the United States,” thus creating less selective pressure.

Tumpey responded that there does not seem to be tremendous selective pressure for H5N1, and that H2 strains that have been isolated from swine need to be considered as potential pandemic threats. [N.B.: an H2 strain was responsible for the “Asian” influenza pandemic of 1957.]
The same audience member asked, “Would a massive stockpile of prepandemic vaccine have any effect on slowing virus spread in future pandemics? What level of enthusiasm will industry have for such a massive stockpile?”

Matthews responded, “How do you choose those prepandemic strains? People are going to want a matched strain in the vaccine, not something like it.” Matthews noted that if we knew the identity of pandemic strains well ahead of time, the main problem of pandemic response would be solved. Shaw noted that “having vaccine stockpiled in a room is one thing; you still need to get the vaccine distributed and administered.”

An audience member asked: “What do you think could have been done better by the media in the coverage of the 2009 H1N1 pandemic?”

Shaw replied, “the media helped a lot and let people become aware of where vaccine was available. They played a good role in that respect. However, they also gave some mixed messages because the media often tries to counterbalance arguments even when the sides are not equal. They did well to convey the message that the vaccine was safe, but I don’t think they got the message across to all the vulnerable populations. The topicality of H1N1 got old quickly. The media coverage needed to continue longer.” Shaw also noted some “holes in the education campaign” and that there was an “abysmal rate of vaccination among health care workers. Health care workers should know better.”

An audience member asked, “When there are case fatalities, are patient samples (tissues) saved in such a way that the genotype of the patient can be obtained in the future? As we learn more about host factors we will need samples to look into patient genotypes.”

Gill responded that samples of tissue obtained at autopsy are frozen or formalin fixed and thus are available for genotyping. He noted that the 1918 pandemic virus had been reconstructed from old army samples and from tissue preserved in permafrost.16 “As far as tissue preservation in cases of H5N1,” Gill adds, “H5N1 is circulating in places where they don’t routinely do autopsies. But, specimens of H5N1 have been collected and are out there.”

An audience member noted the importance of faster and better real-time diagnostics. She asked whether rapid diagnosis in a future, severe pandemic could identify those individuals who had survived infection and could assist in the pandemic response with nonmedical interventions before a vaccine was available. She noted that this knowledge could also help to prioritize distribution of a vaccine to those still susceptible when vaccine supplies are limited.

Shaw responded by saying that better diagnostics are a long-standing goal: “Most of what we have now is antigen based; most of the newer techniques are focusing on nucleic acid detection.” Shaw also noted that nucleic acid detection requires specialized equipment that most physicians do not have in the clinic and that we need to have diagnostics at the bedside—at the point of care. “In my mind nucleic acid detection is about the only way to get a new diagnostic test out there,” said Shaw. He continued by saying that nonmedical interventions can be useful but have drawbacks: “For example, you can give someone a mask, but they may not know how to use it properly, and then, eventually, they get hot and sweaty and the mask comes off. However, there are other situations when nonmedical interventions are useful and should be applied.”

An audience member asked Tumpey and Lowen: “You have different animal models. Can you comment on how the data relate to one another and how the data transfer to humans?”

Tumpey and Lowen responded that there are some differences between the ferret and guinea pig data, differences that may be due to experimental setup or execution: “It is hard to know precisely how the experiments are done in different labs; for example, the airflow may be different.” However, they noted that the similarities between the ferret and guinea pig data are remarkable. Both animal models are naturally susceptible to influenza, with no need for virus adaptation. Tumpey and Lowen concluded, “It is good to have alternative models because animal models are always just models. If the models agree, then it strengthens the data.”

Appendix: Meeting agenda

H1N1 Swine Flu: The 2010 Perspective

Monday, May 24, 2010

Organizers:
Doris Bucher, New York Medical College
Jennifer Henry, the New York Academy of Sciences
2009 H1N1 swine flu: the 2010 perspective

Bucher et al.

12:30 PM
Registration

1:00 PM
Introduction
Doris Bucher, New York Medical College

1:15 PM
Transmission and Pathogenesis of H1N1 Influenza Viruses in Ferrets
Terrence M. Tumpey, Centers for Disease Control and Prevention

1:45 PM
Transmission of the 2009 H1N1 Pandemic Influenza Virus
Anice Lowen, Mount Sinai School of Medicine

2:15 PM
Pathology Findings of Fatal 2009 Influenza A/H1N1 Viral Infections in New York City
James Gill, Office of the Chief Medical Examiner

2:45 PM
Coffee Break

3:15 PM
CDC Pandemic Response: Testing Preparedness
Michael W. Shaw, Centers for Disease Control and Prevention

3:45 PM
The 2009 H1N1 Pandemic—An Industry Perspective
James Matthews, Sanofi Pasteur

4:15 PM
Influenza Virus–Like Particles (VLP) as Effective and Economical Human and Animal Vaccines
Jose M. Galarza, TechnoVax, Inc.

4:45 PM
Panel Discussion (all speakers)
Moderated by Philip R. Dormitzer, Novartis Vaccines and Diagnostics

5:30 PM
Networking Reception

Conflicts of interest

D.B. receives research support from influenza vaccine manufacturers through the organization IFPMA-IVS Taskforce. Three coauthors are employed by manufacturers or developers of influenza vaccines: J.M. (Sanofi Pasteur), J.G. (TechnoVax), and P.D. (Novartis Vaccines and Diagnostics).

References


Genes, brain, and behavior: development gone awry in autism?

A report on the 23rd Annual International Symposium of the Center for the Study of Gene Structure and Function

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Autism and its highly variable symptomology were the themes of the 23rd Annual International Symposium of the Center for the Study of Gene Structure and Function at Hunter College in New York City, held 15 January 2010. The meeting explored the extensive research on autism from several perspectives—integrating research on genetics, neuroscience, and behavior—from researchers presenting new and innovative approaches to understanding the autism spectrum. Early diagnosis, intervention, and genetics were major themes because they are seen as essential areas in which progress is needed before the rise in numbers of cases of autism throughout the world, which some describe as approaching an epidemic, can be stemmed. Several genetic, neurobiological, and behavioral markers of autism have been identified that may ultimately provide the basis for early identification, and that presently define the key areas requiring intensive intervention.

Keywords: autism spectrum disorder (ASD); neuron; synapse; epilepsy; endophenotype; fragile X syndrome (FXS); microcephaly with seizures (MCSZ); mental retardation; connectome; brain circuit; axon; dendrite; Early Start Denver Model (ESDM); mirror neurons; Asperger’s syndrome; specific language impairment (SLI); joint attention

Autism—Integrating Genes, Brain and Behavior, was a day-long symposium held at Hunter College of the City University of New York. It was the 23rd Annual International Symposium of Hunter’s Center for the Study of Gene Structure and Function and was cosponsored by the Clinical and Translational Science Center at Weill Cornell Medical College. The symposium provided an exciting exploration of the basic research on the molecular genetics and neurobiological mechanisms of autism, as well as the social and cognitive research on autism. The speakers, in their respective fields, shared an appreciation for the complexity of autism and the challenges of understanding it. Each speaker offered a unique approach to these challenges using a wide range of research tools and skills. The research and analysis covered ranged from molecular biology and real-time neuronal function to therapeutic early intervention and parental training. The symposium ended with a presentation on the epidemiology of autism; in the context of the data on prevalence, the questions of whether incidence of autism is increasing, and if so, why, were at the forefront of the conference.

The conference, organized by faculty from Hunter College, Weill Cornell Medical College, Memorial Sloan-Kettering Cancer Center, and the Hospital for Special Surgery, originated from discussions between faculty from the Psychology and Biological Sciences Departments at Hunter College. The
presentations were divided into two sections: one focused on cellular and molecular research, while the other focused on behavioral and therapeutic research. However, the two sections were linked by the recognition that progress in our understanding and ability to alleviate the often devastating impact to the family of the autism spectrum disorders will require parallel progress in both areas.

Introduction

Autism spectrum disorders (ASD) comprise a wide range of brain dysfunctions that result in altered social behavior, self-awareness, and language-based interactions. The genetic, biochemical, and cell biological bases of these brain-altered states are only beginning to be understood. Recent focus in these areas of research has been on connecting genes implicated in autism to their function at the synapse, the structure that ensures the transfer of signals from one brain cell (neuron) to the next. Recent data suggest that genes implicated in autism affect the circuitry of the brain, that is, the wiring that defines the particular geography of synaptic connections between neurons. Hence, genes whose products regulate the structure and function of the synapse are likely to be implicated in neuronal wiring and ASD pathogenesis. The idea that many genes may be implicated independently in autism is supported by recent evidence from cases of inherited autism, which occur secondary to multiple, distinct mutations in neuronal genes required for signaling and synapse regulation. The heterogeneous nature of these genetic abnormalities is consistent with the spectrum of phenotypes and severity of altered behaviors that are observed clinically in autism.

In the early stages of brain development, neurons of the central nervous system (CNS) associate to form billions of connections or synapses. While this process is dynamic and just a fraction of these connections persist into adulthood, the retention of important synapses for brain function involves the fine-tuned expression of genes at precise moments in development. Any cellular or physiological errors that disrupt the proper regulation of synapses may cause brain dysfunction. Current research efforts seek to determine if synaptic dysfunction, as seen in autism and related disorders such as fragile X syndrome, contributes to the mis-wiring of brain circuitry. Efforts to link cognitive dysfunction to circuity changes in the brain have focused on many levels, including the structure of dendrites that mediates the presynaptic/postsynaptic connection in the majority of excitatory synapses in the brain, the dendritic spine. Pioneering work of Purpura and Marin-Padilla during the 1970s showed changes in the morphology of synapses in the brains of children with mental retardation.1,2 These changes were characterized by fewer numbers of synapses (i.e., dendritic spines) and greater spine length. A decrease in the number of spines, and thus the number of synapses, has been found in other disease states involving cognition, such as Down syndrome and Alzheimer’s disease.3 A change in the morphology of spines seems to be a unifying theme in diseases of cognition, in that the normal pathway of dendritic spine maturation appears disrupted. This results in longer, thinner spines that resemble immature filopodia, or spine precursors. The challenge now is to link these changes in spine architecture and biochemistry to defects in brain organization, function, and ultimately, behavior.

Autism research has expanded to encompass many of the major behavioral and neurobiological research areas. The recognition that the disorder is not just a problem of social or cognitive development has led to the mobilization of research in genetics, biochemistry, and neurophysiology with behavior, cognition, and development to produce a panoply of research wisdom and skills against what we now know is a spectrum of disorders. The application of research findings from developmental psychology in the areas of perception, cognition, and social skills has provided a foundation upon which autism research has progressed. Likewise the recognition by the autism field of learning and memory processes, and the application of strategies that have emanated from them, have provided insight into the etiology and treatment of affected children. The blend of applied behavioral analysis with cognitive neuroscience approaches has been successfully applied to autism with considerable success. Current autism research employs techniques from discrete trial learning, imitation, language comprehension, and perception, among others. The combinations of these with electrophysiological and imaging methods have linked behavioral and cognitive process affected by autism to discrete brain mechanism. There is recognition that the stages of early developmental processes, with the emergence of behavioral and
physiological changes, have provided the timing for such investigations.

In the morning session, five investigators from around the country highlighted their approaches to autism using a range of genetics and diverse brain imaging approaches. The first cohort of researchers at the symposium discussed recent advances that point to activity-dependent genes that function at the synapse, linking the pre- and postsynaptic compartments. Often, these genes are also master regulators of other genes through control of gene expression at the transcriptional and translational level. The products of these activity-dependent genes may be defective in patients with autism since they are highly heritable, and interestingly, some correlate with language processing function.

In the afternoon session, six other investigators discussed their research. They reported on brain imaging approaches combined with cognitive testing, behavioral intervention strategies, and parent-based support to better diagnose autism earlier and to understand and ameliorate the loss of typical language and joint attention in autism. The symposium concluded with epidemiological research showing possible causes for the increased incidence of autism as characterized today.

The genetics of autism

Dan Geschwind, of the UCLA School of Medicine, opened the conference with a discussion of the “autisms” as a spectrum of developmental behavioral abnormalities that results from a spectrum of genetic disruptions (Fig. 1). While many genes have been implicated in the etiology of ASD, and despite the disease being highly heritable (up to 90% penetrant), the mode of inheritance is not well understood. As many as 25% of patients will develop epilepsy at some point in their life, and many also have intellectual disabilities. It appears to affect males more commonly, at a ratio of 4:1 (males to females) and at an overall rate of 1:150–200 births, being more common than any other childhood disease. While many genes have been implicated, it is important to note that no single gene appears to account for more than 5% of autism cases. Therefore Geschwind’s lab focuses on endophenotypes, or biomarkers of behavioral phenotypes that are stably associated with a genetic component.

Geschwind and colleagues discovered that a single gene mutation co-segregated with a language delay in children with autism, and that this mutation encoded polymorphisms in the contactin-associated protein-like 2 (CNTNAP2) gene.\(^4\) CNTNAP2, a member of the neurexin gene family encoding a trans-synaptic protein involved in synapse formation and maintenance, is expressed in brain regions that are more evolved in primates. Patients with intellectual disabilities, as well as an Amish family with children having focal epilepsy, also carry mutations in this gene; in addition, CNTNAP2 mutations are also highly correlated with...
specific language impairment (SLI), another highly heritable condition that carries no other noticeable developmental abnormalities. Intriguingly, such data connect the CNTNAP2 gene implicated in a highly specific language learning and memory function with autism; more generally, this connection exemplifies the potential of a gene to impact one component on the spectrum of endophenotypes characteristic of a given disorder (such as autism). Given that language impairment is such a prevalent feature of autism, much work has focused on genes that can account for specific language disorders; other genes, such as those encoding the transcriptional control protein FOXP2, are known to cause a monogenic language disorder, although mutations within this gene are rare. This represents another case where gene network interactions highlight a developmentally regulated program to control complex human behaviors. Therefore it is no surprise that the gene encoding CNTNAP2 functions “downstream” of FOXP2 regulation in neurons.5

As a member of the neurexin gene family, CNTNAP2 is one of the best candidates known to link autism with synapses and brain circuits. Neurexins are presynaptic binding partners for postsynaptic proteins termed neuroligins, which have mutated forms in isolated families with multiple autistic family members.6,7 These proteins are important for synaptic formation and maintenance, and are implicated in synapse function and proper brain circuit formation. CNTNAP2 appears to be expressed in highly evolved frontal regions of the human brain and may have played a key role in the evolution of language-based regions in humans (e.g., the anterior perisylvian cortex and the basal ganglia) since this protein is expressed in these areas in non-human primates. Collaborative research between Geschwind’s lab and those of Mirella Dapretto and Susan Bookheimer at UCLA has investigated the proposed role for CNTNAP2 in autism pathogenesis. This research has shown that risk-allele carriers of CNTNAP2 do not display a typical response to externally directed task attention. These patients display a stronger local and weaker long-range connectivity in the frontal-striatal circuits than normal subjects using fMRI techniques, lending credence to the hypothesis of disconnections on a circuit level as a basis for part of the behavioral pathology in autism patients.8

Following the genetic heterogeneity of brain diseases of closely related social groups has been of great value in tracing the origin of autism and other related developmental disorders of intellectual disability, including seizure and mental retardation. Of particular significance are studies of rare recessive mutations that are carried within families for many generations, such as within consanguineous marriages. One such study has been pioneered by Christopher Walsh of Harvard Medical School, who discovered a disorder termed microcephaly with seizures (MCSZ). Children with this disease typically exhibit a reduced head size, developmental delay, and mild seizure disorder. By recognizing a similar phenotype in the offspring of consanguineous marriages, such as in certain Middle Eastern populations, they were able to discover the recessive mutation on chromosome 19 that is responsible for this disorder.9 While the mutations in the separate populations were distinct, they both affected the PNKP gene. How the PNKP gene, which encodes for a DNA repair protein, plays a role in the pathogenesis of MCSZ is not yet known. What is clear is that population-specific mutations in a given gene or genes within the same pathway can give rise to a spectrum of phenotypes depending on the effect of the mutations on protein function.

Using a similar approach to discover the genes involved in autism pathogenesis, Walsh and colleagues formed the Homozygosity Mapping Collaborative for Autism, where clinicians and geneticists in the United States and the Middle East study consanguineous families with autism. They found large copy number variation in a section of chromosome 22, an area previously associated with intellectual disability when mutated.10 Five regions were deleted on both parental sets of chromosomes, impacting six genes, although none were completely disabled in function. These genes include several of unknown function, ionic gating membrane channel proteins, axonal growth factors, and a transcription factor. Significantly, four of the six genes are expressed in the hippocampus, a region of the brain central to learning and memory function, and an area that is already implicated in dysfunction in autism studies. In addition, some of these genes were previously shown to be activated in response to neuronal activity. This activity-dependent response may be a key mechanism to facilitate the proper regulation of synapse firing during experience-dependent
changes in synaptic strength (the cellular correlate of learning and memory). A dysregulation of any of these genes could have direct implications for altered synapse formation and function during brain development.\textsuperscript{5,11}

Further probing the nature of the deletions and the effect on these genes led Walsh and colleagues to use microarray technology, focusing on a single copy number variant that deleted a region near the RNF8 gene, a transcriptional co-regulator.\textsuperscript{10} Transcription factors including RNF8 are proteins that can rapidly switch genes on or off in response to cellular signaling and growth cues. The deletion affected the transcription site of the RNF8 gene in a region that contained a CREB binding site. CREB is another major transcription factor that is mobilized in response to activity to turn on genes that support long-term changes in synaptic strength. Removal of this binding site for CREB will likely have significant effects on RNF8 gene expression and subsequently on the genes that require this cofactor for their expression. Future studies will determine which genes these are and in which direction they are dysregulated in response to the RNF8 mutation.

Taken together, these genetic studies reveal the heterogeneity of causes of autism that parallel the spectrum of observed phenotypes of human behaviors among carriers of these mutations. While the nature of these mutations is only beginning to be revealed, family pedigrees of recessive mutations have implicated genes central to neuronal function, such as those functioning at synapses. A paradigm example of this is the CNTNAP2 gene encoding a trans-synaptic protein mediating synapse formation and function, and its proposed partner neuroligin, which binds to CNTNAP2 family members to bridge pre- and postsynaptic compartments. Loss of function of these genes has direct implications for loss of proper circuit formation, and may lead to synaptic dysconnection during development.\textsuperscript{12} In addition, it appears that seizure disorders such as epilepsy are common to several distinct brain pathologies that involve developmental delay, and therefore this excessive activity may contribute to defects in learning and behavior that manifest in ASD.

**Gene expression, synapses, and circuit formation**

Coming from the other end of the molecular spectrum, Jason Dictenberg of Hunter College is pioneering work on the monogenic causes of autism at the molecular level of the synapse. Fragile X syndrome (FXS) is the leading single-gene cause of autism, presently estimated at ~3–5%. Strikingly ~60% of children with FXS have autistic behavior.\textsuperscript{13} The fragile X protein FMRP, an mRNA-binding protein, is a master regulator of mRNA transport to dendrites. It is also involved in the translation of synaptic genes in response to neuronal activity.\textsuperscript{14} FXS is caused by a trinucleotide(CGG)-repeat expansion in the 5' untranslated region of the FMRP gene that leads to transcriptional silencing secondary to hypermethylation. The mutation is highly heritable and appears to be exaggerated upon generational inheritance. Dictenberg and colleagues use a mouse model of FXS to study synaptic defects in genes implicated in circuit formation and function.

A hallmark of synaptic changes in FXS is the preponderance of long, thin spines that are reminiscent of immature stages during development. Dictenberg and colleagues used a novel RNA-tagging method to demonstrate the role of FMRP in mRNA transport to dendrites in response to neuronal activity. They found that loss of FMRP led to decreased mRNA targeting to dendrites and altered spine morphology.\textsuperscript{15} One example of a specific mRNA affected is the CaMKII-alpha mRNA, which is transported to dendrites in response to glutamatergic signaling to facilitate learning and memory. However, experiments carried out in the mouse model of FXS demonstrated that the processivity (run length) of individual CaMKII-alpha mRNA particles was diminished following metabotropic glutamate receptor (mGluR) signaling. Previous work has shown that, in the absence of FMRP, hippocampal CA1 neurons undergo excessive long-term depression of synaptic responses upon mGluR signaling in the mouse model, a process that normally requires local protein synthesis within dendrites.\textsuperscript{16} Therefore loss of mRNA transport and subsequent dysregulation of local protein synthesis may contribute to defects in synaptic plasticity, learning, and memory function observed in FXS. Dictenberg hypothesizes that a loss of stimulus-induced mRNA transport may lead to precocious mRNA translation before the mRNAs reach the dendrite, with proteins therefore made at the wrong place and time. This mechanism may be common to many of the heterogeneous causes of autism.
Recent data from the Dictenberg lab explores the role of trans-synaptic cell adhesion molecules and their potential dysregulation in FXS. Neuroligins (NLs) are postsynaptic proteins that are implicated in autism pathogenesis in cases of familial inheritance. The proteins are binding partners for the CNTNAP2 family of presynaptic neurexins.\(^6\),\(^7\),\(^17\)

Importantly, Dictenberg made the connection that one of the known mRNA targets of FMRP, PSD-95, is a scaffold protein that can regulate the levels of NLs at synapses.\(^18\) Therefore, dysregulation of PSD-95 expression, as seen in neurons from the mouse model of autism, implicates NL dysfunction. The lab uses super-resolution microscopy methods to quantify changes in proteins at individual synapses upon neuronal activation (Fig. 2). The data show that mGluR stimulation causes a rapid increase (within 15 minutes) in dendritic PSD-95 protein levels in wild-type hippocampal neurons, but that this up-regulation is absent in neurons derived from the FXS mouse model of autism. NL1 protein also appears altered in its expression in FXS neurons, and this altered the ratio of excitatory to inhibitory synapses. Live cell imaging of PSD-95-GFP and NL1-GFP fusion proteins expressed in hippocampal neurons shows dynamic movements within filopodia and spines. Analysis of these protein movements in neurons from FXS mice will lead to a greater understanding of the defects that may give rise to altered plasticity and synapse structure. Future work from the Dictenberg lab aims to determine how an alteration of the balance of excitatory and inhibitory inputs in diverse brain regions contributes to the observed physiological and behavioral changes in FXS and autism, and how excessive activity such as in epilepsy may contribute to synaptic defects and altered circuit architecture.

Looking into the visual system for clues on circuit formation and function, Hollis Cline and her group at Scripps Research Institute turned to the tadpole eye and the innervation pattern of the optic tectum. They used fluorescent proteins...
expressed in these animals and, owing to the relative transparency of these animals, were able to visualize the axonal projections and their changes in connectivity over time. The projections were modified by visual experience as predicted by early experiments by LeVay, Hubel, and Wiesel. These pioneering scientists showed that sensory experience shaped the developing visual cortex during a critical period in brain development and that sensory deprivation led to loss of connectivity in that represented region. Cline used this approach to look into dendritic arborization in response to synaptic activity, a process that is less understood than axonal innervation. Her group showed a dramatic rearrangement of dendritic arbors in less than an hour and, over longer periods, demonstrated an extensive elaboration with almost every process undergoing some change. Through careful analysis this work showed an iterative process whereby branches were elaborated and then subsequently pruned to allow stabilization of only a select few processes until the mature circuit formed. This was consistent with the synaptotrophic hypothesis, whereby synaptic connections control the exploratory behavior of developing neurons, the development of neuronal arbors, and the establishment of neuronal circuits. Visual stimulation was shown to enhance this arborization, and electrophysiological experiments showed the strengthening of existing synapses and the addition of new synapses as well, further confirming the validity of the hypothesis.

Cline’s lab observed not only these changes in neuronal excitability and arborization upon sensory stimulation, but also modification of gene expression. In searching for an activity-dependent mechanistic cause to explain these broad-ranging effects of visual stimulation on the brain, her group looked to mRNA-binding proteins like FMRP that control mRNA stability, transport, and translation. They focused on CPEB, which binds to cytoplasmic polyadenylated mRNA tails to control their subsequent translation upon neuronal activity. Using a dominant-negative form of the CPEB protein that interferes with normal CPEB function, they looked to the effects on arborization in the tadpole visual circuit. The mutant protein diminished the overall growth rate of the dendrites and the branch length, which dampened the circuit formation.

Together, the studies of Cline and Dictenberg highlight the role of gene expression in controlling synapse formation, structure and function, and how synaptic activity can alter gene expression to modify synaptic inputs and the topography of the overall circuit. Cline’s elegant work highlights the plasticity of the circuit with regard to sensory input and dendritic morphology, and shows how this can affect the brain on a macroscopic level over longer periods of time.

The connectome

Bringing the theory of genomics to the study of synaptic connections, Jeffrey Lichtman of Harvard University discussed the architecture of the wiring of human brains and suggested that most diseases of the nervous system could be a result of aberrant effects on the circuitry of neurons. The work of his lab aims to build on the observations of the early neuroscientists Golgi and Ramon y Cajal, who discovered the interconnectivity of the neurons in the brain and speculated on the directionality of information flow from one neuron to the next within a circuit. The wiring of individual neurons within the human brain is astoundingly complex. Therefore Lichtman’s lab uses advances in imaging technologies to better assess connectivity. Similar to the way that geneticists have mapped out the genome, his group seeks to map the human “connectome” to have a working diagram of an apparently normally wired brain. He acknowledged that “normally wired” may be inaccurate, since genes are expressed uniquely within individuals despite sharing over 99% genetic similarity. These individual expression patterns may affect neuronal connections so that each person has refined connections that are uniquely based on experience-dependent learning and plasticity. Despite these limitations, Lichtman’s group has advanced the connectome project by applying computer-assisted image acquisition and analysis to the structural mapping of sets of neuronal circuits and the nervous system as a whole. Once the connectome is mapped, withstanding some accepted diversity in the wiring, this knowledge could be applied to animal models of neurological diseases, like autism, to determine the defects in wiring architecture that may give rise to altered learning, memory, and behavior.

Lichtman’s group has shown differences in the developmental connectivity of mouse brain using the neuromuscular junction (NMJ) as a model system. The NMJ is where the motor nerves that originate in
the spinal cord innervate muscle peripherally. Early after birth there exist multiple neurons (axons) that contact the same muscle fiber junction.\textsuperscript{22} After a few weeks in mice (and months in humans), a pruning of these connections takes place such that eventually only a single axon innervates the junction. The other axons retract and find other targets, resulting in a smaller motor unit in the fiber that is stable throughout the lifetime of the animal.\textsuperscript{23} This process of multiple innervation followed by pruning during development occurs in the central nervous system as well, and the mechanisms for this process appear to be similar.

To better follow the individual connections of axons with the muscle fibers during development, the Lichtman lab used spectrally distinct fluorescent protein markers to visualize individual fibers. Using time-lapse imaging methods, they observed \textit{in vivo} the initial innervation and synaptic competition, subsequent synaptic formation, and finally synapse retraction during development of the mouse. Expanding on this approach, they created a new line of transgenic mouse that expresses three different spectral varieties of fluorescent proteins within the brain. By driving the expression of each color (blue, green, and red) randomly within each cell, they were able to create a “brainbow” mouse where each cell has a distinct color variation from the next.\textsuperscript{24} Given the complexity and density of packing of individual fibers within the central nervous system, this technique serves to disambiguate each neuronal process even within the context of hundreds or thousands of neighboring processes (Fig. 3). Using these mice, Lichtman discovered that an axonal connection at the NMJ that is dominant, or one that occupies most of the junctional space, may not always win out over the lesser-connected axons. The neuron of the “winning” axon, however, was always observed to be successful at adjacent synapses where another branch of its axon innervated a distinct NMJ.\textsuperscript{25} These results suggest that the “successful” neuron is able to express synaptic molecules that enable it to strengthen its synapses more efficiently than other competing neurons in response to activity at the NMJ.

Applying new technological advances in tissue sectioning along with the fluorescent labeling technique, Lichtman’s group was able to demonstrate the highly individualized nature of axonal innervation by mapping the connectome of the interscutularis muscle of the mouse ear. Using a serial section technique that preserves a continuous connection between subsequent slices, they generated a strip of tissue that spans the whole muscle, leaving its neuronal processes intact. Upon examination of the

![Figure 3. Connectomics with no tracing necessary! Brainbow mouse sections of the peripheral nervous system neuromuscular junction (NMJ) highlight the use of randomized fluorescent protein expression within each neuron. Individual axons can be distinguished from each other as they enter the muscle (left) and at the synapse (right) using this technique as they innervate the NMJ during development. Slide courtesy of Jeff Lichtman.](image-url)
connectivity, they found that, strikingly, between genetically identical animals, the patterns were distinct (i.e., like the distinct fingerprints of identical twins). This individuality included arbor shapes, branching patterns, and the number of branches. Also noticed was a suboptimal pattern of connectivity, with branches often passing a connection before looping back to make a synapse, which Lichtman says is evidence that the connectome is to some degree built “on the fly” without pre-programming. He believes that the patterning of synapse elimination “unfetters the mammalian nervous system from the tyranny of the genes.” In the end, each animal ends up with a very different nervous system, largely due to environmental influences and experiences, through basic learning and memory mechanisms. Ultimately, connectomics research may allow investigators to compare brains of autistic patients to normal individuals to determine which circuits are disrupted and how this correlates with behavioral differences.

**New directions in early detection and intervention in autism**

The second keynote address was presented by Geraldine Dawson (Fig. 4), chief science officer of Autism Speaks, an autism research and advocacy organization. She provided an overview of the research on early diagnosis of ASD and clearly articulated the consensus in the field that there is a need to accelerate the identification and treatment of ASD. She pointed out that while there have been deficiencies of the health care system in recognizing the need for early diagnosis, there are now several early childhood screening options, including some that appear to be useful in identifying signs of autism as early as six months. In agreement with the speakers of the morning session, Dawson indicated the importance of the genetic components of ASD and the fact that siblings of autistic children are at much higher risk for the disorder. This provides an opportunity for prospective research on these infants that may assist with the detection of signs of autism even earlier.

Dawson and others have shown that up to six months of age, children who will go on to develop autism typically seem fairly normal. Deficits in attention and selective engagement with human faces and voices begin to appear at this age and increase in severity to one year when the social and communication deficits of autism can clearly be identified.

![Figure 4. Geraldine Dawson, keynote speaker.](image-url)
in most ASD children. It is important to note, however, that between a quarter and a third of children who develop ASD do not show deficits until one or two years of age.

Dawson described innovative research that shows promise for recognizing the early signs of autism. First, she discussed research indicating that high-risk infants have diminished ability to distinguish lightness and darkness in the visual field and that this deficit may play a role in the inability to respond to human faces.28 Next, Dawson presented ground-breaking research identifying deficits in event-related potentials (ERPs) to human faces in a specific cortical region, the fusiform gyrus, of high-risk infants. The deficit is characterized as diminished amplitude in this electrophysiological pattern that is not lateralized to the right hemisphere as it is in low-risk infants.29 She states that the challenge is to determine whether these and other promising findings are predictive of the disease or whether they are endophenotypes indicating a predisposition to the disease.

Early intervention has been shown to have an impact on learning and language development of ASD. Dawson asserts that along with the successful strategies from applied behavior analysis and discrete trials training, the use of methods from developmental psychology that take advantage of the “intuitive” learning of children as they actively explore their environment might produce the most effective intervention strategy.30 This thinking emphasizes comprehensive approaches to intervention and is at the heart of Dawson’s collaboration with Sally Rogers in the Early Start Denver Model (ESDM), a comprehensive intervention program for young children with autism. The ESDM Program is a collaborative effort of experts—physicians, psychologists, behavioral therapists, speech pathologists, and occupational therapists—who work to intervene on as many ASD deficits as possible. Social development with language, motor, and cognitive skills are intensively applied to autistic children. She states, “We’re building a baby from the ground up in all senses of the word.” Their promising findings31 using this approach have encouraged Dawson, Rogers, and their team to expand the program to other sites. They believe that programs that bring together therapists, experts, and parents in early intervention offer the greatest promise of improvement for those with ASD.

Social motivation, attention, and learning in the autistic brain

Early in development, infants preferentially focus their attention on human faces and voices—particularly the face and voice of their mother—while infants with ASD do not show this preference. Dawson discussed how the ERP response to faces is diminished in autistic children. The fusiform “face area” and the amygdala, a key brain region involved with emotion, exhibit reduced amplitude ERP responses in children with ASD.

Mirella Dapretto, at the University of California, Los Angeles, and colleagues have investigated these findings from the perspective that autism may involve the alteration of brain function in areas that mediate “theory of mind,” or the interpretation of others’ intentions, actions, and emotional states. Her research has shown that although deficits in the brain regions associated with theory of mind are observed when unfamiliar faces are presented to autistic children,32 the response is normative when familiar faces are presented. The deficit appears to be associated with attentional mechanisms: when autistic children are instructed to pay attention during testing, they exhibit normal levels of activity in these brain areas.33

With this evidence that brain function is essentially intact in these areas, Dapretto shifted her focus to explore the lack of preference for facial and vocal stimuli in ASD. “According to the social motivation hypothesis, this lack of an attentional preference for these stimuli may reflect that they’re not ‘rewarding’,” she said. These stimuli typically produce reinforcement in unaffected children but not in children with ASD. Her research showed that social and nonsocial reinforcement that activates the brain reward regions, such as the ventral striatum, fails to do so in children with ASD. The difference was particularly striking with social rewards.

These deficits suggest that brain centers that mediate emotion, especially emotion generated in social situations, may be impaired. Dapretto and colleagues have performed experiments in which ASD children were instructed to mimic facial expressions that show different emotions. Although the children performed well on the task behaviorally, functional magnetic resonance imaging
(fMRI) shows that the children exhibited deficits in important brain regions in comparison to unaffected children (Fig. 5). In particular, they showed lower levels of activity in brain areas with “mirror neurons.” These are regions of the brain that are thought to be necessary for following and interpreting movements and intentions of others, and may be key to understanding social and emotional situations. Dapretto’s research is consistent with others’ in suggesting that this system may be impaired in autistic children. Dapretto indicated that there may be a link between the deficits in mirror neuron activity and attentional bias seen with autism; recent data from her lab indicates that there is a lack of coordination between the reward system and mirror neuron centers with ASD. According to Dapretto, children who have highest activity in the ventral striatum (when they were getting the smiling faces as positive feedback) were also the children who showed greater activity in those mirroring regions that are thought to be important for interpreting another’s facial expression and feelings. Dapretto concluded by stating that the social and emotional impairments observed with ASD may be due to deficits in the mechanisms that mediate long-range integration between functioning brain regions. This could be exacerbated by impaired attentional bias. Her current research is focused on the role these differences may play in the deficits observed with the development of linguistic abilities.

Language in ASD: from behavioral phenotypes to neurobiology and genetics

Language deficits are a common feature of ASD. Exploring the range of these deficits is the focus of work by Helen Tager-Flusberg (Boston University) and her colleagues. They have examined the differences of those who have intact language skills (autism language normal; ALN), who may still have diminished abilities to understand social cues and the embedded meaning of speech (often labeled Asperger’s syndrome) compared with those with more severe language deficits (autism language impaired; ALI). ALI children were found to exhibit deficits that are surprisingly similar to those diagnosed with SLI on language tests. Tager-Flusberg and colleagues showed that ALN did not show these deficits. With these findings as a clue, she and her colleagues explored the brain regions that mediate language to determine if the deficits produced identifiable anomalies. She found that ALI children show impairments in the left hemisphere lateralization of speech. Typically, the development of language is associated with increased left hemisphere dominance of Broca’s area in the frontal cortex (the speech production area) and Wernicke’s area in the temporal cortex (the speech recognition area). Autistic children exhibit little left hemisphere dominance or even reversed dominance of Broca’s area concomittant with an exaggerated left
dominance in Wernicke’s area. Tager-Flusberg and colleagues found that this profile was associated with ALI children, and not ALN children. She and her colleagues investigated other language regions, exploring the arcuate fasciculus, the major pathway connecting Broca’s and Wernicke’s areas.\(^\text{37}\) Using diffusion tensor imaging of the arcuate fasciculus, they found no apparent differences between ADS children and unaffected controls. However, when ASD children were divided on the basis of linguistic ability, it was apparent that ALI showed diminished connectivity between the two speech areas.

Siblings of autistic children are at greater risk for developing autism, and they exhibit reduced language performance and similar alterations of brain language centers as their affected siblings.\(^\text{38}\) This was exemplified by Tager-Flusberg and colleagues’ work in which they found that siblings showed lower language and reading performance and similar anomalies of Broca’s and Wernicke’s areas as ASD children. Since not all of these siblings will go on to have ASD, it suggests that the language impairments and altered brain systems that mediate language represent an endophenotype for ASD.

Tager-Flusberg also reported preliminary data on identification of basic speech sounds at ages six months and nine months as they relate to autism. With normal development, there is a change in recognition of these sounds associated with greater brain lateralization at the later stage of development. Infants at risk for ASD exhibited no change in recognition and a lack of asymmetry in brain development at age nine months. These data suggest that there may be a seven- to nine-month period during which developmental abnormalities can be detected and followed as indicators of risk for the development of ASD. Identification of genetic markers that are associated with the language deficits during this period may lead to markers useful in identifying risk for ASD. A finding such as that reported by Geschwind earlier in the symposium indicating a mutation in the CNTNAP2 gene that is passed on maternally and is associated with language development is very encouraging and is being pursued by Tager-Flusberg and colleagues.

**Integrating neuropsychology, development, behavior, and treatment**

Sally Rogers, at the University of California, Davis, described how we have come to understand the essential features of autism. We now understand that ASD is manifested differently in early childhood in comparison to school age. She described how the various cognitive impairments of affected two to five year olds can be grouped in “clusters” of social, emotional, action-oriented, linguistic, perceptual, and learning deficits.

In the 1990s, autism research emphasized a model in which the symptoms of autism were seen as a function of the impact of biological and environmental factors on cognitive processes. Therefore, treatment strategies tended to selectively target the problem areas with the hope that there would be improvements in symptoms associated with them. Along these lines, strategies that teach imitation to ASD children have been shown to improve symbolic play, joint attention, and language skills.

More recently, an alternative model has gained favor: one that ties the symptoms of ASD to dysfunctions in the structure and connectivity of specific brain regions. Rogers reflected on Geschwind’s earlier presentation as an example of this approach. There is a growing body of information that ASD is associated with a diminished capacity for the formation of long-range circuits that are essential for the integration of complex brain functions. She believes that these circuits are essential for the development of complex skills that are deficient in ASD. In addition, she described a different potential problem associated with the development of these critical circuits. As discussed in Lichtman’s presentation in the morning session, there may be deficits in the formation of brain circuits associated with a lack of “pruning” of local brain circuits that result in over-connectedness within these systems, thereby leading to disorganization at the local level. Rogers believes that interventions that include teaching imitation behavior provide the stimulation for the development of efficient circuits. There is evidence that when engaging in these tasks, ASD children (even the high functioning individuals) show diminished and more localized brain activity than their unaffected peers. She believes that early learning of these and other skills is the key to producing the essential neuronal circuits. Early interventions can bolster the development of neuronal circuits that are probably less robust in those with ASD. Although time consuming, the repeated exercises of discrete trial training and other behavioral methods of applied
A series of other approaches, particularly those that include naturalistic teaching, show great promise. Allowing children to determine the choice and focus of activities in the context of daily programs with adults leads to the development of important skills and is similar to what parents do with their children. In this context, Rogers explored her research with Dawson on the ESDM.31 Using a video presentation of an ESDM training session, she illustrated the range of situations and reinforcements employed by the program. Social interactions, including eye contact, motor activity, and affirming emotional feedback, provide effective reinforcement that is evident. The focus is primarily on the various neuropsychological deficits of the early stages of ASD. The hope is that active play in the context of the developmental training program will lead to the acquisition of skills that then permits cognitive and social advancement, which may in turn allow them to catch up to their peers. She presented additional data from a University of Washington study described by Dawson showing that the percentage of children that met diagnostic criteria for intellectual disability in the sample decreased from 70% to 30% after two years of the program. She concludes that these findings are evidence of the potency of early learning and experiences in the acquisition of cognitive, social, and motor skills. Early intervention with ESDM and similar programs that emphasize an intensive “integrative” approach will prove invaluable in the development of the essential neural networks in young ASD children and lead to better treatment.

A parent-mediated intervention

The establishment of joint attentional focus between infant and adult is essential for the development of early language acquisition. Michael Siller at Hunter College views early language learning as a collaborative process in which a child and adult both attend to the same object or task in parallel. The basic processes associated with this state are quite powerful and the failure of their development may be a major factor in the language deficits in autism. Siller’s research suggests that the deficits in joint attention, which are common in ASD children, make it exceedingly difficult for children to effectively pair words with their meanings. Typically, children between 15 and 19 months are able to recognize the focus of another person’s attention, and as a result label the object. Before this age, language as well as other learning depends on the parental establishment of joint attention. “What you hear a lot is parents’ labeling objects to which the child is already attending, or commenting on actions or intentions or goals that the child is pursuing,” he said.

Siller, in collaboration with Marian Sigman at UCLA, has shown that a child’s capacity for joint attention correlates directly with their subsequent acquisition of language skills.39 Siller and Sigman described how joint attention deficits are observed in children with ASD, probably disrupting language development. Longitudinal studies by Siller and Sigman also suggested that autistic children benefit from a process sometimes used by mothers with their autistic children, a process called maternal “synchronization.” With synchronization, the mother continually uses engaging language to talk about and describe the objects and actions of a child’s attention, thereby mitigating their language deficits.

Recently Siller and colleagues have investigated the processes that are the basis of the benefits of synchronization and have been developing an intervention program to train parents of ASD children in the effective use of responsive language with their children. Data from his recently completed randomized controlled trial indicate that an intervention designed to effectively train individuals to use these techniques could be implemented and prove effective in stimulating language development in autistic children. The program involves 12 in-home training sessions in which both parent–child and interventionist–child interactions are employed. Conventional teaching, live modeling, and coaching methods are used in the program. Each session is recorded on video and subsequently reviewed, enabling parents and interventionists to review progress and make adjustments to improve the success of the intervention.

Siller reported on a pilot study to aid in the development of the study guide for the intervention involving a small sample. His team partnered with the California Regional Centers to recruit a larger sample of families from the Los Angeles metropolitan area for a randomized controlled trial. Children in the trial had a clinical diagnosis of ASD, severe language impairment, and were six years of age or younger. The group using the training showed improvements in the use of maternal synchronization over their performance at the beginning.
of the study, thus indicating that the intervention procedures were effective in training parents in the use of responsive language. Further examination of the data is required to determine if there were long-term gains in language resulting from this enhanced synchronization. Siller and colleagues are currently analyzing the first year follow-up data. He is also collaborating with Connie Kasari at UCLA to explore the possibility of a similar intervention with younger children (18 to 30 months old).

**Epidemiology and the changing paradigm of ASD**

The concluding presentation given by Marshalyn Yeargin-Allsopp provided a review of ongoing efforts of the CDC to determine the prevalence of ASD in the United States and provide information on the factors that contribute to changes in these statistics (Fig. 6). She began by listing some of the factors that have confounded previous efforts to accurately measure the incidence of autism. Most of these confounds are related to the quality of the measurement criteria, including quantification of onset based on diagnosis at a relatively late age (averaging 4 to 6 years old), failure to confirm diagnoses over the course of a study, and the use of inaccurate or outdated diagnostic standards.

This latter factor is a crucial consideration in current attempts to measure prevalence. Yeargin-Allsopp provided a brief update on the change in criteria. This change is especially important considering that autism was first described in 1956 by Leo Kanner and that in 1980 this disorder was reclassified as a developmental disability rather than a mental illness. Other milestones are the inclusion of ASD in the World Health Organization’s ICD-10 in the 1990s and in the American Psychiatric Association’s DSM-IV, both of which considerably broadened the description of ASDs and included high-functioning individuals such as those with Asperger’s syndrome. She indicated that an increase in prevalence has occurred in parallel with the use of the broader, more nuanced diagnostic criteria. This has made it difficult to understand the changes in prevalence over time, which have been dramatic: increasing from 4–5 in 10,000 individuals prior to 1990s standards to 6 cases per 1,000 individuals more recently. Yeargin-Allsopp stated that prevalence trends are limited by the changes in the characterization of the illness, frequently leading to contradictory interpretations.

Yeargin-Allsopp’s team at the CDC uses a multiple source record review in their efforts to accurately determine trends. Using this strategy to monitor ASD along with other developmental disorders, they found that the prevalence among eight-year-olds in the five counties of metropolitan Atlanta showed a prevalence of 6.5 per 1000 in the year 2000. These data were surprising because its peak prevalence exceeded all disorders being studied, including diseases such as cerebral palsy, hearing loss, or visual impairment.

In light of such statistics, the U.S. Congress expanded the funding of this program and the Autism and Developmental Disabilities Monitoring (ADDM) Network to now encompass multiple sites across the nation. Surveillance at all locations

![Figure 6. Summary and historical perspective on autism prevalence before 2009, as assessed by the CDC (Yeargin-Allsopp).](image-url)
incorporates records from multiple health and education sources, with all cases subject to review and confirmation by clinicians using DSM-IV criteria. “Our results have really become the standard for setting ASD prevalence estimates for the United States,” said Yeargin-Allsopp.

Surveillance data from 14 different sites for the year 2002 monitored nearly 10% of American children for ASD. The average prevalence was 6.6 per 1,000—a measurement that is the basis for the 1:150 statistic that was cited throughout the symposium. The conclusion, based on this rate of prevalence, is that approximately 560,000 Americans under the age of 21 are affected by the disorder. In December 2010, the CDC reported on data collected from 11 sites in 2006 that indicated an even-higher prevalence of 1 in 110 children, a 57% increase since 2002. As with previous surveys, the disparity between boys and girls remains approximately the same—boys are more than four times more likely to be diagnosed with ASD than girls. These data are consistent with those of Europe and Asia. The current criteria for development of ASD include onset by the age of three; however, the average age of diagnosis of the disorder is considerably later at four and one-half years of age. In agreement with many of the other presentations, Yeargin-Allsopp stated, “We obviously still have a lot of work to do in terms of identifying children with these behaviors early.”

Conclusion

Having Marshalyn Yeargin-Allsopp of the CDC conclude the symposium put ASD in the context of the national debate. The CDC data and other sources around the world suggest an epidemic of ASD among various demographic groups across the United States. In the past few decades, the number of children with ASD has gone from around 4 cases per 10,000 children to as high as 1 in 150 in some areas. Although the precise reasons for this precipitous rise are unclear, previous efforts to accurately measure autism were flawed, and the definition of those included on the spectrum has recently broadened considerably. She concluded that fully exploring and addressing this problem will require a concerted effort among scientists, clinicians, and governmental and non-governmental agencies. New detection methods now show potential to identify high-risk infants as early as 6 months of age. The detection of changes in language recognition with development in typical children was notably absent in autistic children, and a novel paradigm of maternal “synchronization” with an ASD child’s attention was shown to be effective in stimulating language development. A new understanding of the genetic abnormalities that may contribute to the cause of autism was highlighted, with both large mutations (such as with copy number variations that delete the RNF8 gene) and single gene mutations (such as with the fragile X gene and the contactin-associated protein, CNTNAP2) playing a significant role in the etiology of ASD. In addition, the function of these genes in the formation of circuits, which are being mapped in the human brain currently, is an exciting area of research that is just beginning to open up new possibilities for treatment. The fact that epilepsy is a highly comorbid factor with ASD suggests that excessive excitatory activity during brain development may contribute to the defects in learning, behavior, and brain wiring observed in ASD; this must be addressed in future studies using anticonvulsant drugs to determine which types of ASD may respond favorably. The presenters and attendees of the symposium benefited from the knowledge exchange at the intersection of cell biology, neuroscience, developmental psychology, and public health on the understanding and treatment of autism. Sharing the results in these disparate fields should fuel new and exciting research that may one day alleviate the pain and suffering experienced by patients and families affected by autism.

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