The Wistar Institute of Anatomy and Biology

Annual Progress Report: 2006 Nonformula Grant

Reporting Period

July 1, 2010 – May 31, 2011

Nonformula Grant Overview

The Wistar Institute of Anatomy and Biology received $4,206,096 in nonformula funds for the grant award period June 1, 2007 through May 31, 2011. Accomplishments for the reporting period are described below.

Research Project: Project Title and Purpose

Development of a Universal Influenza A Virus Vaccine - The purpose of this project is to generate a universal influenza A virus vaccine that induces protective immunity to all influenza A virus strains, including newly evolving pandemic strains. Current vaccines to influenza A virus induce protection mainly through neutralizing antibodies directed against surface proteins (antigens) of influenza virus. Since the viral surface antigens are constantly undergoing mutations, each year new vaccines have to be developed that are tailored to the newly evolved influenza A virus strains. A universal influenza A virus vaccine would work against all strains of the virus and hence reduce the need for annual vaccination campaigns. Furthermore, a universal influenza A virus vaccine would dramatically increase our overall preparedness for the next influenza A virus pandemic.

Duration of Project

6/1/2007 - 5/31/2011

Project Overview

Influenza virus vaccines protect through induction of subtype specific antibodies against surface antigens of the virus. These antigens constantly mutate and vaccines thus have to be redesigned and reapplied annually. Vaccines against a new pandemic strain cannot be produced in advance, as the molecular composition of this strain cannot be predicted. The goal of this proposal is to generate a novel universal influenza virus vaccine that induces broad protection against all strains of the virus. We will test 5 hypotheses: 1) $CD8^+$ T cells to conserved internal proteins, such as the nucleoprotein (NP) and the matrix (M) protein, and antibodies to the ectodomain of the matrix 2 protein (M2e) of influenza A virus protect against diverse strains of the virus. 2) $CD8^+$ T cells and M2e-specific antibodies act synergistically and vaccines that induce both responses provide superior protection than vaccines that induce only one type of response. (3) Inactivated vaccines and infections with influenza A virus do not induce sufficiently potent cross-reactive immune responses to protect against a heterotypic infection. 4) Novel subunit

vaccines based on adenovirus vectors can induce protective cross-reactive T cell responses; and 5) adenovirus vectors expressing M2e can induce protective antibodies to M2e; such responses may be enhanced by a prime-boost regimen with M2e peptides. The proposal has 4 specific aims. We will develop vaccines based on replication-defective chimpanzee adenovirus vectors expressing NP, M or M2e and M2e peptides. Viral vector and peptide vaccines will be tested individually and then in combination for their ability to induce cross-reactive immune responses in aim 1 and for protection against challenge with different strains of influenza A virus in mice and ferrets in aim 2. We will test human subjects for cross-reactive immunity to influenza A virus strains in aim 3 to provide baseline data for future clinical trials with a universal influenza A virus vaccine. Cohorts will include subjects that are highly susceptible to influenza virus, that have been exposed to avian strains, and that are acutely infected or convalescent. Sera from volunteers will be tested for neutralizing antibodies and for antibodies to M2e. Peripheral blood mononuclear cells will be tested for T cells to NP and M. The analyses will show to what degree the magnitude and diversity of neutralizing antibodies to different strains of influenza A virus correlate with titers of antibodies to M2e and T cells to NP and M. Analyzing antibody titers to H5 and H7 will show if cohorts with reported exposure to birds have an increased prevalence of such antibodies. We will develop a cocktail of human monoclonal antibodies to M2e for treatment of acute influenza A virus infections in aim 4.

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Other Participating Researchers

Andrew J. Caton, PhD, Jan Erikson, PhD, Walter Gerhard, MD. Luis J. Montaner, DVM, DPhil, E. John Wherry, PhD, William H. Wunner, PhD - employed by The Wistar Institute of Anatomy and Biology. (Dr. Wherry is as of June 2010 employed by the University of Pennsylvania)
Michael R. Betts, PhD, Sherrill Davison, VMD, Sarah J. Ratcliffe, PhD, Guido Silvestri, PhD - employed by University of Pennsylvania
Susan Coffin, MD, MPH - employed by Children's Hospital of Philadelphia
Caroline C. Johnson, MD - employed by City of Philadelphia
Laszlo Otvos, PhD - employed by Temple University

Expected Research Outcomes and Benefits

We plan to develop a novel universal vaccine against influenza A viruses. A universal vaccine has to first show efficacy in animals and then safety and efficacy in humans. Once it is marketed, this novel vaccine will benefit all Pennsylvanians (and others) by reducing their vulnerability to influenza A virus infections including those caused by emerging pandemic strains. It will eliminate the routine need for annual vaccination against new variants of influenza A viruses and

it will remove the uncertainties associated with the selection of strains of influenza A virus that are incorporated each year into the vaccine. If the universal vaccine shows the expected efficacy in animals and humans, we would aim to license the technology to a Pennsylvania-based pharmaceutical company, which would benefit the Pennsylvania economy by providing new jobs and taxable sales revenues for the company. We will also develop a cocktail of monoclonal antibodies for early treatment of influenza A virus infections. This too would benefit Pennsylvanians, especially in the case of a pandemic where anti-viral drug supplies may rapidly become limited or circulating influenza A viruses may become drug-resistant. We will analyze different cohorts of Pennsylvanians for cross-reactive T and B-cell responses to influenza A virus, including those who are highly susceptible to influenza A virus infection, such as young children and those with demonstrated recent infections. This will facilitate the design of future clinical trials by providing baseline data about levels of responses that an efficacious universal vaccine will have to exceed. We will compare these to cohorts that experience frequent exposures to poultry, including those with documented exposures to birds that were infected with avian strains of influenza virus during previous outbreaks in Pennsylvania, to determine the likelihood of cross-over infections. This cohort will also serve as a valuable sentinel cohort in case a new avian strain of influenza A virus that can potentially infect humans reaches the birds population of Pennsylvania.

Summary of Research Completed

PROJECT 1

Generation of AdC68 Vectors
Since our last report, we generated an additional vector prototype in which M2e is expressed within VR1 of hexon, and three versions of M2e fused to NP are encoded by an expression cassette within the Ad vectors E1 domain.

AdC Vaccine-Induced Influenza A Virus-Specific Immune Responses and Protection Against Challenge
The prime-boost vaccine was tested in a ferret challenge study. Ferrets were protected from death and there was a modest decrease in viral titers on day 5 after challenge.

We generated vectors expressing M2e of A/PR8 within VR1 or VR4 of AdC68. M2e placed within VR1 showed superior binding of M2e-specific monoclonal antibodies and elicited higher antibody responses to M2e (Figure 1).

Antibody titers to M2e were measured one month later.

In an additional permutation we generated an AdC68 vector expressing M2e within VR1 of hexon, which also encoded the Me2-NP fusion protein from a cassette placed within E1. This vector induced superior antibody responses to the original vector (which only encoded the fusion protein, Figure 2), and it induced T cells to NP (not shown).

PROJECT 2

Long-lived antibody secreting cells (ASCs) are critical for protective humoral immunity, but the mechanisms governing their development and maintenance are poorly understood. We have examined the longevity and survival requisites of respiratory tract ASCs in contrast to ASCs found at other sites in response to influenza virus infection. Irradiation of mice at various time points after influenza virus infection significantly diminishes lung ASCs, suggesting that they are short-lived and may require extrinsic factors for their persistence. Recent studies have emphasized the significance of the TNF superfamily members BLyS (B lymphocyte stimulator, also termed BAFF) and APRIL (a proliferation-inducing ligand) as key regulators of ASC survival. BLyS and APRIL bind two receptors, TACI (transmembrane activator and calcium inducer) and BCMA (B cell maturation antigen), while BLyS also binds a third receptor, BR3 (BLyS receptor 3). Neutralization of BLyS and APRIL with TACI-Fc reduced anti-viral ASC numbers in lungs and bone marrow, whereas ASCs in spleen and lung-draining lymph node were surprisingly unaffected. TACI$^{-/-}$ mice mounted initial anti-viral B cell responses similar to WT mice, but they failed to sustain protective Ab titers in the airways and serum, leading to increased susceptibility to secondary viral challenge. These studies highlight the importance of TACI signaling for the maintenance of anti-viral ASCs and protection to influenza virus infection. A manuscript has been submitted detailing these findings. (Wolf A. I., Mozdzanowska K., Quinn III W., Metzgar M., Williams K., Caton A.J., Meffre E., Bram R., Allman D., Cancro M. P., and Jan Erikson (2011). Protective anti-viral B cell responses in the respiratory tract require TACI. *Under review in The Journal of Clinical Investigation)*

Having established that the M2eMaps can induce protective immunity, we engineered the M2e-MAP vaccine to contain T helper epitopes that have been reported to be promiscuous with the goal to generate a vaccine able to be used in a genetically diverse population. They include epitopes from circumsporozoite protein (CS) of *Plasmodium falciparum*, hepatitis B virus antigen (HBsAg) and influenza virus hemagglutinin (HA). The M2e- MAP peptides are named K2 and K3. To test whether the K2 and K3 peptide vaccine regimen shows efficacy in mouse strains other than BALB/c mice (H-2^{d}), we extended our studies to C57BL/6 (H-2^{b}) and C3H (H-2^{k}) mice, as well as the out bred mouse strains CD1/ICR and Swiss Webster. Intranasal immunization of (4) M2e-MAP K2 in combination with adjuvants was unable to elicit a significant anti-M2e Ab response and failed to induce protection against challenge with X31 virus (data not shown). To test whether out bred mouse strains are able to mount a response to M2e following influenza virus infection, we used the previously reported scheme of multiple infections that has been shown to elicit small but significant levels of anti-M2e Abs in BALB/c mice. In 3-4 weeks intervals, BALB/c mice and the out bred mouse strains Swiss Webster and CD1 ICR were infected first with PR8 virus, then with PR8-Seq14, which evades HA-based neutralization, and thirdly X31. Both out bred strains elicited a strong anti-PR8 serum IgG response (Figure 3A). In contrast to BALB/c mice, Swiss Webster and CD1 ICR mice had reduced levels of anti-M2e Abs in their serum (Figure 3B). We next tested whether the inability of strains to mount a sufficient M2e Ab response after vaccination was due to a failure in T cell activation to the T cell epitopes included in the vaccine. We assayed the proliferative response of cells in the draining lymph node after subcutaneous immunization. Cells from immunized BALB/c mice, but not C57BL/6 or C3H mice, proliferated upon restimulation with peptide in a dose-dependent manner (Figure 3C).

To further investigate the genetic restrictions, we next immunized C57BL/6xBALB/c (F1). As shown in Figure 3D, intranasal immunization with K2 and adjuvants elicited high anti-M2e-Ab titers against both peptide and native M2. The use of allotype-specific reagents Igh*a* for BALB/c and Igh*b* for C57BL/6 revealed that B cells independent of the strain background were capable of producing anti-M2e Abs (Figure 3E). To examine whether the levels of anti-M2e Abs induced in vaccinated C57BL/6xBALB/c (F1) mice were sufficient to provide protection, mice were challenged with X31 virus and viral titers determined. As compared to control-immunized mice, vaccination with K2 peptide resulted in significantly reduced viral titers in the lungs (Figure 3F). The results suggest that C57BL6 mice do not have a hole in the B cell repertoire that would prevent them from recognizing and responding to the (4) M2e-MAP.

In conclusion, immunization of other inbred and out bred mouse strains with the M2e-MAP vaccine resulted in poor humoral and T cell responsiveness and failed to protect mice against challenge. Although synthetic MAPs are promising designs for vaccines, these results highlight the need to improve promiscuous T helper epitopes to increase MAP efficacy in a genetically diverse group. This data will be presented in a manuscript that is currently in preparation: Wolf A. I., Mozdzanowska K., Otvos L., Hoffmann R., Singer D., Gerhard W. and Jan Erikson (2011). An improved M2e-targeted peptide vaccine confers protection against influenza virus infection.

PROJECT 3

Influenza virus-specific T cell responses in different cohorts of human subjects

Adults exposed to avian influenza virus strains**:** Human subjects from the poultry industry in Lancaster County and elsewhere in Pennsylvania where avian influenza outbreaks have occurred in birds were identified. With the help of our colleagues, Sherrill Davidson and Susan Casavant at the University of Pennsylvania School of Veterinary Medicine, blood was obtained from 45 subjects and T cell analysis performed.

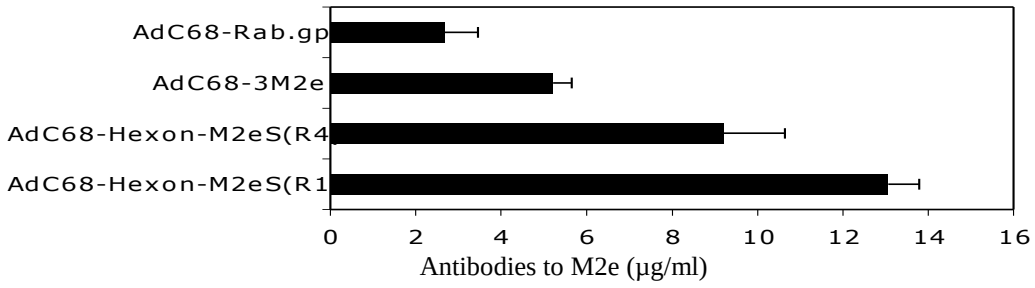

Figure 1. Mice were immunized with an Ad vector expressing rabies virus glycoprotein (AdC68Rab.gp) and Ad vector encoding three versions of M2e (AdC68-3M2), an Ad vector expressing M2e within VR4 (AdC68-Hexon-M2eS(R4)) or VR1 (AdC68-Hexon-M2eS(R1) of hexon.

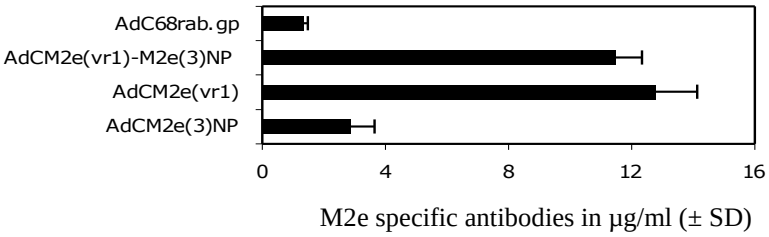

Figure 2: Young C57Bl/6 mice were immunized with 10^{10} vp of AdC68M2e(3)NP, AdC68M2e(vr1) or AdC68Me2(vr1)-M2e(3)NP. They were bled two weeks later, and antibody titers to M2e were measured by a cellular ELISA against a standard. Graph shows titers in µg/ml ± SD. Differences in titers of AdC68M2e(3)NP and AdC68M2e(vr1) or AdC68M2e(vr1)-M2e(3)NP are highly significant ($p<0.01$).

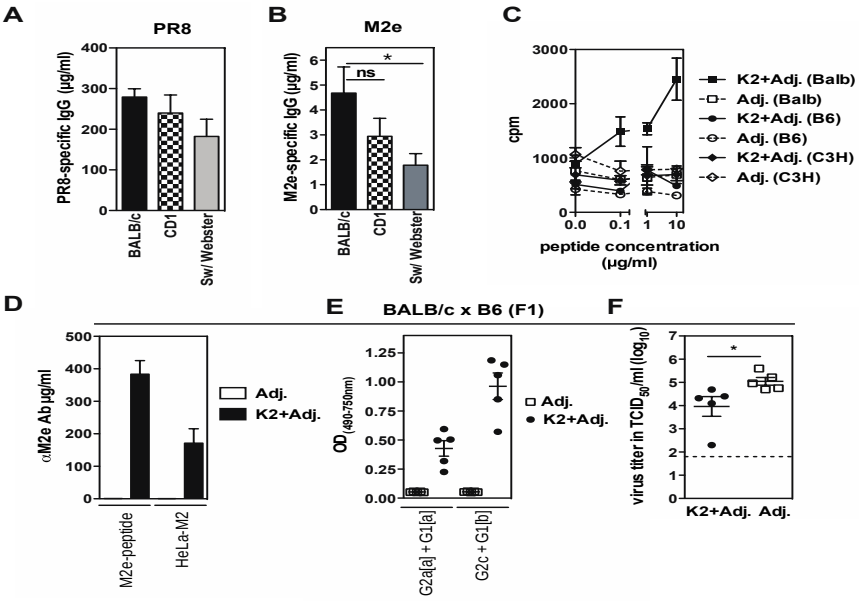

Figure 3. **(A and B)** BALB/c, CD1 ICR and Swiss Webster mice (n=5-6 mice) received three consecutive infections, first with influenza virus A/PR8, then PR8-SEQ14 and finally with X31. PR8-specific IgG serum Abs **(A)** and M2e-specific IgG **(B)** were determined after third infection. **(C)** BALB/c, C57BL/6, and C3H mice were immunized s.c. with K2 peptide and adjuvants, and cellular proliferation was assessed by incorporation of 3H-thymidine during the fourth day of *in vitro* culture in presence of various concentrations of K2 peptide. One out of two independent experiments shown. **(D-F)** C57BL/6xBALB/c (F1) mice (n=5 mice) were immunized i.n. with K2+Adjuvants or adjuvants alone. **(D)** M2e-specific Ab titers measured in an ELISA against M2e-peptide or HeLa-M2 cells in serum of C57BL/6xBALB/c (F1) mice after third immunization. **(E)** M2e-specific Serum Ab titers determined with allotype-specific reagents (IgG2a[a] and IgG1[a] for BALB/c and IgG2c and IgG1[b] for C57BL/6 origin of Abs). **(F)** After the third immunization, C57BL/6xBALB/c (F1) mice were challenged with influenza virus X31 (1000 $TCID_{50}$/5µl), and infectious virus in the lungs was determined five days post challenge.