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<td>Phenotypic heterogeneity of antigen-specific CD4 T cells under different conditions of antigen persistence and antigen load</td>
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<td>Pathogenicity and immunogenicity of influenza viruses with genes from the 1918 pandemic virus</td>
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Hepatitis C Virus and HIV Envelope Proteins Collaboratively Mediate Interleukin-8 Secretion through Activation of p38 MAP Kinase and SHP2 in Hepatocytes

Hepatitis C and Human Immunodeficiency Virus Envelope Proteins Cooperatively Induce Hepatocytic Apoptosis via an Innocent Bystander Mechanism

Presence of Human Immunodeficiency Virus-1-Specific CD4 and CD8 Cellular Immune Responses in Children with Full or Partial Virus Suppression
BLOOD 100 (2002) 1381–1387
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Production of a p55gag Particle Vaccine Using the Baculovirus Expression Vector System Technology

Treatment of primary HIV-1 infection with cyclosporin A coupled with highly active antiretroviral therapy

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Apoptosis in asymptomatic HIV-1 seropositives immunized with HIV-1 env glycoprotein (gp160): effects of administration of Zidovudine in vivo and interleukin-2 in vitro

Immune responses elicited by recombinant vaccinia-human immunodeficiency virus (HIV) envelope and HIV envelope protein: analysis of the durability of responses and effect of repeated boosting

Induction of humoral and cell-mediated anti-human immunodeficiency virus (HIV) responses in HIV sero-negative volunteers by immunization with recombinant gp160
Dissolved Carbon Dioxide Determines the Productivity of a Recombinant Hemagglutinin Component of an Influenza Vaccine Produced by Insect Cells

Jamal Meghrous¹, Nikolai Khramtsov¹, Barry C. Buckland¹,², Manon M.J. Cox¹, Laura A. Palomares¹,³, Indresh K. Srivastava¹

¹ Protein Sciences Corporation, 1000 Research Parkway, Meriden, CT 06450, USA
² University College London, London, United Kingdom
³ Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Mexico

ABSTRACT

Dissolved carbon dioxide (dCO2) accumulation during cell culture has been recognized as an important parameter that needs to be controlled for successful scale-up of animal cell culture because above a certain concentration there are adverse effects on cell growth performance and protein production. We investigated the effect of accumulation of dCO2 in bioreactor cultures of expresSF² insect cells infected with recombinant baculoviruses expressing recombinant influenza virus hemagglutinins (rHA). Different strategies for bioreactor cultures were used to obtain various ranges of concentrations of dCO2 (<50, 50–100, 100–200, and >200 mmHg) and to determine their effects on recombinant protein production and cell metabolic activity. We show that the accumulation of dCO2 at levels >100 mmHg resulted in reduced metabolic activity, slowed cell growth, prolonged culture viability after infection, and decreased infection kinetics. The reduced rHA yields were not caused by the decrease in the extracellular pH that resulted from dCO2 accumulation, but were most likely due to the effect of dCO2 accumulation in cells. The results obtained here at the 2 L scale have been used for the design of large-scale processes to manufacture the rHA-based recombinant vaccine Flublok™ at the 2500 L scale.


The baculovirus expression vector system: A commercial manufacturing platform for viral vaccines and gene therapy vectors

Felberbaum, Rachael S.¹

¹ Protein Sciences Corporation, 1000 Research Parkway, Meriden, CT 06450, USA

ABSTRACT

The baculovirus expression vector system (BEVS) platform has become an established manufacturing platform for the production of viral vaccines and gene therapy vectors. Nine BEVS-derived products have been approved – four for human use (Cervarix®, Provenge®, Glybera® and Flublok®) and five for veterinary use (Porcilis® Pesti, BAYOVAC CSF E2®, Circumvent® PCV, Ingelvac CircoFLEX® and Porcilis® PCV). The BEVS platform offers many advantages, including manufacturing speed, flexible product design, inherent safety and scalability. This combination of features and product approvals has previously attracted interest from academic researchers, and more recently from industry leaders, to utilize BEVS to develop next generation vaccines, vectors for gene therapy, and other biopharmaceutical complex proteins. In this review, we explore the BEVS platform, detailing how it works, platform features and limitations and important considerations for manufacturing and regulatory approval. To underscore the growth in opportunities for BEVS-derived products, we discuss the latest product developments in the gene therapy and influenza vaccine fields that follow in the wake of the recent product approvals of Glybera® and Flublok®, respectively. We anticipate that the utility of the platform will expand even further as new BEVS-derived products attain licensure. Finally, we touch on some of the areas where new BEVS-derived products are likely to emerge.
Titer on chip: new analytical tool for influenza vaccine potency determination

Kuck LR\textsuperscript{1}, Sorensen M\textsuperscript{2}, Matthews E\textsuperscript{2}, Srivastava I\textsuperscript{2}, Cox MM\textsuperscript{2}, Rowlen KL\textsuperscript{1}

\textsuperscript{1} Protein Sciences Corporation, 1000 Research Parkway, Meriden, CT 06450, USA; Department of Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, UK
\textsuperscript{2} Protein Sciences Corporation, 1000 Research Parkway, Meriden, CT 06450, USA

ABSTRACT

Titer on Chip (Flu-ToC) is a new technique for quantification of influenza hemagglutinin (HA) concentration. In order to evaluate the potential of this new technique, a comparison of Flu-ToC to more conventional methods was conducted using recombinant HA produced in a baculovirus expression system as a test case. Samples from current vaccine strains were collected from four different steps in the manufacturing process. A total of 19 samples were analysed by Flu-ToC (blinded), single radial immunodiffusion (SRID), an enzyme-linked immunosorbent assay (ELISA), and the purity adjusted bicinchoninic acid assay (paBCA). The results indicated reasonable linear correlation between Flu-ToC and SRID, ELISA, and paBCA, with regression slopes of log-log plots being 0.91, 1.03, and 0.91, respectively. The average ratio for HA content measured by Flu-ToC relative to SRID, ELISA, and paBCA was 83\%, 147\%, and 81\%, respectively; indicating nearly equivalent potency determination for Flu-ToC relative to SRID and paBCA. These results, combined with demonstrated multiplexed analysis of all components within a quadrivalent formulation and robust response to HA strains over a wide time period, support the conclusion that Flu-ToC can be used as a reliable and time-saving alternative potency assay for influenza vaccines.

FIGURE 1: The immunoassay is illustrated in the left panel and the array layout is outlined in the right panel. The array contains 9 replicate spots (~200 µm in diameter) of each monoclonal antibody (designated A–I).
Technology transfer and scale-up of the Flublok® recombinant hemagglutinin (HA) influenza vaccine manufacturing process

Buckland B\textsuperscript{a,c}, Boulanger R\textsuperscript{a}, Fino Mireli\textsuperscript{a}, Srivastava I\textsuperscript{a}, Holtz K\textsuperscript{a}, Khramtsov N\textsuperscript{a}, McPherson C\textsuperscript{b}, Meghrous J\textsuperscript{a}, Kubera P\textsuperscript{a}, Cox MM\textsuperscript{a}

\textsuperscript{a} Protein Sciences Corporation, 1000 Research Parkway, Meriden, CT 06450, USA
\textsuperscript{b} ABEC Corporation, Bethlehem, PA, USA
\textsuperscript{c} Department of Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, UK

ABSTRACT
Multiple different hemagglutinin (HA) protein antigens have been reproducibly manufactured at the 650L scale by Protein Sciences Corporation (PSC) based on an insect cell culture with baculovirus infection. Significantly, these HA protein antigens were produced by the same Universal Manufacturing process as described in the biological license application (BLA) for the first recombinant influenza vaccine approved by the FDA (Flublok\textsuperscript{®}). The technology is uniquely designed so that a change in vaccine composition can be readily accommodated from one HA protein antigen to another one. Here we present a vaccine candidate to combat the recently emerged H7N9 virus as an example starting with the genetic sequence for the required HA, creation of the baculovirus and ending with purified protein antigen (or vaccine component) at the 10L scale accomplished within 38 days under GMP conditions. The same process performance is being achieved at the 2L, 10L, 100L, 650L and 2500L scale. An illustration is given of how the technology was transferred from the benchmark 650L scale facility to a retrofitted microbial facility at the 2500L scale within 100 days which includes the time for facility engineering changes. The successful development, technology transfer and scale-up of the Flublok\textsuperscript{®} process has major implications for being ready to make vaccine rapidly on a worldwide scale as a defense against pandemic influenza. The technology described does not have the same vulnerability to mutations in the egg adapted strain, and resulting loss in vaccine efficacy, faced by egg based manufacture.

Mechanism of a Decrease in Potency for the Recombinant Influenza A Virus Hemagglutinin H3 Antigen During Storage

Hickey JM, Holtz KM, Manikwar P, Joshi SB, McPherson CE, Buckland B, Srivastava IK, Middaugh CR, Volkin DB

ABSTRACT
The recombinant hemagglutinin (rHA)-based influenza vaccine Flublok\textsuperscript{®} has recently been approved in the United States as an alternative to the traditional egg-derived flu vaccines. Flublok is a purified vaccine with a hemagglutinin content that is threefold higher than standard inactivated influenza vaccines. When rHA derived from an H3N2 influenza virus was expressed, purified, and stored for 1 month, a rapid loss of in vitro potency (50%) was observed as measured by the single radial immunodiffusion (SRID) assay. A comprehensive characterization of the rHA protein antigen was pursued to identify the potential causes and mechanisms of this potency loss. In addition, the biophysical and chemical stability of the rHA in different formulations and storage conditions was evaluated over time. Results demonstrate that the potency loss over time did not correlate with trends in changes to the higher order structure or hydrodynamic size of the rHA. The most likely mechanism for the early loss of potency was disulfide-mediated cross-linking of rHA, as the formation of non-native disulfide-linked multimers over time correlated well with the observed potency loss. Furthermore, a loss of free thiol content, particularly in specific cysteine residues in the antigen’s C-terminus, was correlated with potency loss measured by SRID.
Integrating Risk Management into Computer System Validation | IVT

Timothy Fields
Protein Sciences Corporation, Meriden, Connecticut, USA

ABSTRACT

The last decade has brought about a number of changes to how pharmaceutical companies address validation. These changes have been brought about primarily by regulatory changes and the economy. Rather than focusing on the “documented evidence” aspect of validation, companies and regulators are switching the focus to “where is the biggest risk” and managing thusly. In 2005, the International Conference on Harmonization (ICH) issued ICH Q9 Quality Risk Management and followed it up with ICHQ10 Quality Management Systems (2). These two documents along with ICH Q8 Pharmaceutical Development set the stage for using a risk-based approach to validation. In 2011, the European Commission revised Annex 11 to the European Union (EU) Good Manufacturing Practices (GMPs) to increase the focus on risk. The shift in focus to use of a risk-based approach and management responsibility should result in more appropriate validation efforts rather than “paperwork.”

Evaluation of safety and immunogenicity of recombinant influenza hemagglutinin (H5/Indonesia/05/2005) formulated with and without a stable oil-in-water emulsion containing glucopyranosyl-lipid A (SE+GLA) adjuvant

SOURCE: University of Rochester School of Medicine, Rochester, NY, United States.

ABSTRACT

Background: Alternatipression of recombinant hemagglutinin (rHA) in insect cells represents a technology with proven efficacy in seasonal influenza and with the potential for a rapid response to the emergence of new, pandemic strains. We evaluated the safety and immunogenicity of rHA vaccine (H5/Indonesia/5/05) produced in SF+ insect cells using a baculovirus expression vector system (BEVS). The rHA vaccine was tested with and without the adjuvant glucopyranosyl lipid A/stable emulsion (GLA/SE).

Methods: Healthy adults 18-49 were randomized to two IM doses on Days 0 and 21 of placebo; unadjuvanted rHA 135μg or 45μg, or rHA 45μg, 15μg, 7.5μg or 3.8μg with GLA/SE. A pioneer group was monitored through Day 42 before randomizing remaining subjects. H5-specific antibody was determined by hemagglutination inhibition (HAI) and microneutralization (MN) on Days 0, 21 and 42.

Results: 392 subjects were randomized, of whom 380 (97%) received two doses and 386 (98%) completed 12 months of follow-up. Injection site pain and tenderness were seen in 50-70% of rHA+GLA/SE recipients and 4-9% of rHA alone and placebo recipients, but most complaints were mild to moderate in intensity. After two doses, the proportions of subjects with HAI titers ≥1:40 were 32% and 15% in the unadjuvanted 135μg and 45μg groups, and 82%, 75%, 66%, and 72% in those receiving 45μg, 15μg, 7.5μg, or 3.8μg with GLA/SE. The geometric mean titers (GMTs) of HAI antibody on Day 42 were 128, 95, 69, and 72 in the 45μg, 15μg, 7.5μg, or 3.8μg with GLA/SE groups, respectively.

Conclusions: rHA GLA/SE was well tolerated and immunogenic in healthy adults, and GLA/SE substantially improved the serum antibody response. rHA expressed using BEVS recombinant DNA platform technology represents a promising strategy for pandemic control.
Pandemic influenza vaccine: characterization of A/California/07/2009 (H1N1) recombinant hemagglutinin protein, insights into H1N1 antigen stability

Protein Sciences Corporation, Meriden, Connecticut, USA

ABSTRACT

Background: The recent H1N1 influenza pandemic illustrated the shortcomings of the vaccine manufacturing process. The A/California/07/2009 H1N1 pandemic influenza vaccine or A(H1N1)pdm09 was available late and in short supply as a result of delays in production caused by low yields and poor antigen stability. Recombinant technology offers the opportunity to shorten manufacturing time. A trivalent recombinant hemagglutinin (rHA) vaccine candidate for seasonal influenza produced using the baculovirus expression vector system (BEVS) was shown to be as effective and safe as egg-derived trivalent inactivated vaccine (TIV) in human clinical studies. In this study, we describe the characterization of the A/California/07/2009 rHA protein and compare the H1N1 pandemic rHA to other seasonal rHA proteins.

Results: Our data show that, like other rHA proteins, purified A/California/07/2009 rHA forms multimeric rosette-like particles of 20-40 nm that are biologically active and immunogenic in mice as assayed by hemagglutination inhibition (HAI) antibody titers. However, proteolytic digest analysis revealed that A/California/07/2009 rHA is more susceptible to proteolytic degradation than rHA proteins derived from other seasonal influenza viruses. We identified a specific proteolytic site conserved across multiple hemagglutinin (HA) proteins that is likely more accessible in A/California/07/2009 HA, possibly as a result of differences in its protein structure, and may contribute to lower antigen stability.

Conclusion: We conclude that, similar to the recombinant seasonal influenza vaccine, recombinant A(H1N1) pdm09 vaccine is likely to perform comparably to licensed A(H1N1)pdm09 vaccines and could offer manufacturing advantages.

Adjuvant solution for pandemic influenza vaccine production

Christopher H. Clegg\textsuperscript{a,1}, Richard Roque\textsuperscript{a}, Neal Van Hoeven\textsuperscript{b}, Lucy Perrone\textsuperscript{a}, Susan L. Baldwin\textsuperscript{b}, Joseph A. Rininger\textsuperscript{c}, Richard A. Bowen\textsuperscript{d}, and Steven G. Reed\textsuperscript{b, e}

\textsuperscript{a} TRIA Bioscience Corp., Seattle, Washington, USA
\textsuperscript{b} Infectious Disease Research Institute, Seattle, Washington, USA
\textsuperscript{c} Protein Sciences Corporation, Meriden, Connecticut, USA
\textsuperscript{d} Department of Biomedical Sciences, Colorado State University, Fort Collins, Colorado, USA
\textsuperscript{e} Immune Design Corp., Seattle, Washington, USA

ABSTRACT

Extensive preparation is underway to mitigate the next pandemic influenza outbreak. New vaccine technologies intended to supplant egg-based production methods are being developed, with recombinant hemagglutinin (rHA) as the most advanced program for preventing seasonal and avian H5N1 Influenza. Increased efforts are being focused on adjuvants that can broaden vaccine immunogenicity against emerging viruses and maximize vaccine supply on a worldwide scale. Here, we test protection against avian flu by using H5N1-derived rHA and GLA-SE, a two-part adjuvant system containing glucopyranosyl lipid adjuvant (GLA), a formulated synthetic Toll-like receptor 4 agonist, and a stable emulsion (SE) of oil in water, which is similar to the best-in-class adjuvants being developed for pandemic flu. Notably, a single submicrogram dose of rH5 adjuvanted with GLA-SE protects mice and ferrets against a high titer challenge with H5N1 virus. GLA-SE, relative to emulsion alone, accelerated induction of the primary immune response and broadened its durability against heterosubtypic H5N1 virus challenge. Mechanistically, GLA-SE augments protection via induction of a Th1-mediated antibody response. Innate signaling pathways that amplify priming of Th1 CD4 T cells will likely improve vaccine performance against future outbreaks of lethal pandemic flu.
Randomized clinical trial of immunogenicity and safety of a recombinant H1N1/2009 pandemic influenza vaccine containing AdvaxTM polysaccharide adjuvant

David L. Gordon\textsuperscript{a}, Dimitar Sajkov\textsuperscript{b}, Richard J. Woodman\textsuperscript{d}, Yoshikazu Honda-Okubo\textsuperscript{f}, Manon M.J. Cox\textsuperscript{e}, Susanne Heinzel\textsuperscript{f}, Nikolai Petrovsky\textsuperscript{b, c, f, *}

\textsuperscript{a} Department of Microbiology and Infectious Diseases, Flinders Medical Centre & Flinders University, Adelaide, Australia
\textsuperscript{b} Australian Respiratory and Sleep Medicine Institute, Flinders Medical Centre & Flinders University, Adelaide, Australia
\textsuperscript{c} Department of Endocrinology, Flinders Medical Centre & Flinders University, Adelaide, Australia
\textsuperscript{d} Discipline of General Practice, Flinders University, Australia
\textsuperscript{e} Protein Sciences Corporation, Meriden, Connecticut, USA
\textsuperscript{f} Vaxine Pty Ltd, Flinders Medical Centre, Adelaide, Australia

ABSTRACT

Background: Timely vaccine supply is critical during influenza pandemics. A recombinant hemagglutinin (rHA)-based vaccine could overcome production hurdles of egg-based vaccines but has never previously been tested in a real-life pandemic setting. The primary aim was to determine the efficacy of a recombinant pandemic vaccine and whether its immunogenicity could be enhanced by a novel polysaccharide adjuvant (AdvaxTM).

Methods: 281 adults aged 18–70 years were recruited in a randomized, subject and observer blinded, parallel-group study of rHA H1N1/2009 vaccine with or without adjuvant. Immunizations were at 0 and 3 weeks with rHA 3, 11 or 45 g. Serology and safety was followed for 6 months.

Results: At baseline, only 9.1% of subjects (95% CI: 6.0–13.2) had seroprotective H1N1/2009 titers. Seroconversion rates varied by rHA dose, presence of adjuvant, subject age and number of immunizations. Eighty percent (95% CI: 52–96) of 18–49 year olds who received rHA 45 g with adjuvant were seroprotected at week 3, representing a 11.1-fold increase in antibody titers from baseline. AdvaxTM adjuvant increased seroprotection rates by 1.9 times after the first, and 2.5 times after the second, immunization when compared to rHA alone. Seroprotection was sustained at 26 weeks and the vaccine was well tolerated with no safety issues.

Conclusions: The study confirmed the ability to design, manufacture, and release a recombinant vaccine within a short time from the start of an actual influenza pandemic. AdvaxTM adjuvant significantly enhanced rHA immunogenicity.

Recombinant protein vaccines produced in insect cells

Manon M. J. Cox, Protein Sciences Corporation, Meriden, Connecticut, USA

ABSTRACT

The baculovirus-insect cell expression system is a well known tool for the production of complex proteins. The technology is also used for commercial manufacture of various veterinary and human vaccines. This review paper provides an overview of how this technology can be applied to produce a multitude of vaccine candidates. The key advantage of this recombinant protein manufacturing platform is that a universal “plug and play” process may be used for producing a broad range of protein-based prophylactic and therapeutic vaccines for both human and veterinary use while offering the potential for low manufacturing costs. Large scale mammalian cell culture facilities previously established for the manufacturing of monoclonal antibodies that have now become obsolete due to yield improvement could be deployed for the manufacturing of these vaccines. Alternatively, manufacturing capacity could be established in geographic regions that do not have any vaccine production capability. Dependent on health care priorities, different vaccines could be manufactured while maintaining the ability to rapidly convert to producing pandemic influenza vaccine when the need arises.
Characterization of a Recombinant Influenza Vaccine Candidate Using Complementary LC-MS Methods

Hongwei Xie¹*, Catalin Doneanu¹, Weibin Chen¹, Joseph Rininger² and Jeffery R. Mazzeo¹
¹ Department of Biopharmaceutical Sciences, Waters Corporation, 34 Maple Street, Milford, Massachusetts, USA
² Protein Sciences Corporation, 1000 Research Parkway, Meriden, Connecticut, USA

ABSTRACT

Influenza vaccination is recognized as the most effective method for reducing morbidity and mortality due to seasonal influenza. To improve vaccine supply and to increase flexibility in vaccine manufacturing, cell culture-based vaccine production has emerged to overcome limitations of egg-based production. The switch of production system and the need for annual re-evaluation of vaccines for the effectiveness due to frequent viral antigenic changes call for methods for complete characterization of the hemagglutinin (HA) antigens and the final vaccine products. This study describes advanced liquid chromatography-mass spectrometry (LC-MS) methods for simultaneous identification of HA proteins and process-related impurities in a trivalent influenza candidate vaccine, comprised of purified recombinant HA (rHA) antigens produced in an insect cell-baculovirus expression vector system (BEVS). N-linked glycosylation sites and glycoforms of the three rHA proteins (corresponding to influenza A subtypes H1N1 and H3N2 and B virus, respectively) were profiled by peptide mapping using reversed-phase (RP) LC-MSE (data independent acquisition LC-MS using an alternating low and elevated collision energy scan mode). The detected site-specific glycoforms were further confirmed and quantified by hydrophilic interaction LC (HILIC)-multiple reaction monitoring (MRM) assays. LC-MSE was used to characterize the vaccine candidate, providing both protein identities and site-specific information of glycosylation and degradations on each rHA protein. HILIC-MRM methodology was used for rapid confirming and quantifying site-specific glycoforms and potential degradations on each rHA protein. These methods can contribute to the monitoring of vaccine quality especially as it pertains to product comparability studies to evaluate the impact of production process changes.

Protocol: Evaluation of the Virus Counter® for rapid baculovirus quantitation

Matthew M. Ferris¹*, Patricia C. Stepp¹, Kirk A. Ranno¹, Wafaa Mahmoudb, Elizabeth Ibbitsonb, James Jarvisb, Manon M.J. Coxb, Kurt Christensenb, Heather Votawc, Dean P. Edwardsb, Kathy L. Rowlena
¹ InDevR, Inc., 2100 Central Ave., Suite 106, Boulder, Colorado, USA
² Protein Sciences Corporation, 1000 Research Parkway, Meriden, Connecticut, USA
³ Baylor College of Medicine, Baculovirus/Monoclonal Antibody Facility, Dan L. Duncan Cancer Center, Houston, Texas, USA

ABSTRACT

The utility of a new instrument for rapid virus quantitation, the Virus Counter, was evaluated in a blind study conducted at three sites. This instrument is a substantially improved version of the original academic research instrument described previously by Stoffel and Rowlen (2005a). The addition of hydrodynamic focusing, a self-contained fluidics system and customized software for system control and data analysis has resulted in a commercially viable and available design. Baculovirus samples were provided by Protein Sciences Corporation and blinded to InDevR and Baylor College of Medicine. Protein Sciences Corporation and Baylor College of Medicine analyzed the samples by plaque assay and InDevR analyzed the samples using the Virus Counter. Serial dilution of stock viruses into growth media and buffer allowed for comparison of measured versus intended concentrations. Direct log-scale comparison between pooled Virus Counter results and pooled plaque assay results indicated a linear relationship (slope = 1.1±0.2, R² = 0.86) with statistically significant Pearson correlation (r = 0.93, p < 0.001).
Influenza Viral Neuraminidase: The Forgotten Antigen

Bert E. Johansson¹ and Manon M. J. Cox²
¹ Division Of Pediatric Critical Care Medicine, El Paso Children's Hospital, El Paso, Texas, USA
² Protein Sciences Corporation, Meriden, Connecticut, USA

ABSTRACT
Influenza is the most common cause of vaccine preventable morbidity and mortality, despite the availability of the conventional trivalent inactivated vaccine (TIV) and the live attenuated vaccine (LAIV). These vaccines induce an immunity dominated by the response to hemagglutinin (HA) and are most effective when there is sufficient antigenic relatedness between the vaccine strain and the circulating wild-type virus' HA. Vaccine strategies against influenza may benefit from inclusion of other viral antigens in addition to HA. Epidemiologic evidence and studies in animals and humans indicate that anti-neuraminidase (NA) immunity will provide protection against severe illness or death in the event of a significant antigenic change in the HA component of the vaccine. However, there is little NA immunity induced by TIV and LAIV. The quantity of NA in influenza vaccines is not standardized and varies significantly among manufacturers, production lots, and tested strains. The activity and stability of the NA enzyme is influenced by concentration of divalent cations. If immunity against NA is desirable, a better understanding of how the enzymatic properties affect the immunogenicity is needed.

A fast track influenza virus vaccine produced in insect cells

Manon M. J. Cox, Yoshifumi Hashimoto
Protein Sciences Corporation, Meriden, Connecticut, USA

ABSTRACT
The viral surface protein hemagglutinin (HA) has been recognized as a key antigen in the host response to influenza virus in both natural infection and vaccination because neutralizing antibodies directed against HA can mitigate or prevent infection. The baculovirus-insect cell system can be used for the production of recombinant HA molecules and is suitable for influenza vaccine production where annual adjustment of the vaccine is required. This expression system is generally considered safe with minimal potential for growth of human pathogens. Extensive characterization of this novel cell substrate has been performed, none of which has revealed the presence of adventitious agents. Multiple clinical studies have demonstrated that the vaccine is safe, well tolerated and immunogenic. The baculovirus-insect cell system could, therefore, be used for the expedited production of a safe and efficacious influenza vaccine. As a result, this technology should provide a fast track worldwide solution for newly emerging influenza strains or pandemic preparedness within a few years.

Letter to the Editor:
An initiative to manufacture and characterize baculovirus reference material

Amine A. Kamen¹, Marc G. Aucoin², Otto-Wilhelm Merten³, Paula Alves⁴, Yoshifumi Hashimoto⁵, Kari Airenne⁶, Yu-Chen Hu⁷, Mauro Mezzina⁸, Monique M. van Oers⁹
¹ National Research Council, Montreal, Canada
² University of Waterloo, Canada
³ Généthon, France
ABSTRACT
This letter to the editor brings to the attention of researchers an initiative to develop a baculovirus reference material repository. To be successful, this initiative needs the support of a broad panel of researchers working with baculovirus vectors for recombinant protein production and gene delivery for either therapy or vaccination. First there is a need to reach a consensus on the nature of the reference material, the production protocols and the baculovirus characterization methods. It will also be important to define repository and distribution procedures so that the reference material is available to any researcher for calibrating experimental data and to compare experiments performed in the various laboratories. As more and more baculovirus-based products are licensed or in the final stages of development, the development of a repository of baculovirus reference material is timely. This letter describes the requirements for the reference material and for the project as a whole to be successful and calls for a partnership that would involve academic, industrial laboratories and governmental organizations to support this international initiative.


Protective efficacy of a trivalent recombinant hemagglutinin protein vaccine (FluBlok®) against influenza in healthy adults: A randomized, placebo-controlled trial

John J. Treanor*a, Hana El Sahlyb, James Kingc, Irene Grahamd, Ruvim Iziksone,
Robert Kohbergerf, Peter Patriarca, Manon Coxg

a University of Rochester, Rochester, New York, USA
b Baylor College of Medicine, Houston, Texas, USA
c University of Maryland, Baltimore, Maryland, USA
d St. Louis University, St. Louis, Missouri, USA
e Protein Sciences Corporation, Meriden, Connecticut, USA
f Blair & Co, Greenwich, Connecticut, USA
g Biologics Consulting Group, Inc., Bethesda, Maryland, USA

ABSTRACT
Background: Development of influenza vaccines that do not use embryonated eggs as the substrate for vaccine production is a high priority. We conducted this study to determine the protective efficacy a recombinant, baculovirus-expressed seasonal trivalent influenza virus hemagglutinin (rHA0) vaccine (FluBlok®).

Methods: Healthy adult subjects at 24 centers across the US were randomly assigned to receive a single injection of saline placebo (2304 subjects), or trivalent FluBlok containing 45 mcg of each rHA0 component (2344 subjects). Serum samples for assessment of immune responses by hemagglutination-inhibition (HAI) were taken from a subset of subjects before and 28 days after immunization. Subjects were followed during the 2007–2008 influenza season and combined nasal and throat swabs for virus isolation were obtained from subjects reporting influenza-like illness.

Results: Rates of local and systemic side effects were low, and the rates of systemic side effects were similar in the vaccine and placebo groups. HAI antibody responses were seen in 78%, 81%, and 52% of FluBlok recipients to the H1, H3, and B components, respectively. FluBlok was 44.6% (95% CI, 18.8%, 62.6%) effective in preventing culture-confirmed influenza meeting the CDC influenza-like illness case definition despite significant antigenic mismatch between the vaccine antigens and circulating viruses.

Conclusions: Trivalent rHA0 vaccine was safe, immunogenic and effective in the prevention of culture-confirmed influenza illness, including protection against drift variants.
Evaluation of the safety, reactogenicity and immunogenicity of FluBlok® trivalent recombinant baculovirus-expressed hemagglutinin influenza vaccine administered intramuscularly to healthy adults 50–64 years of age

R. Baxter\textsuperscript{a,}\textsuperscript{*}, P.A. Patriarca\textsuperscript{b}, K. Ensor\textsuperscript{c}, R. Izikson\textsuperscript{c}, K.L. Goldenthal\textsuperscript{d}, M.M. Cox\textsuperscript{c}

\textsuperscript{a} Kaiser Permanente Vaccine Study Center, Oakland, California, USA
\textsuperscript{b} Biologics Consulting Group, Inc., Bethesda, Maryland, USA
\textsuperscript{c} Protein Sciences Corporation, Meriden, Connecticut, USA
\textsuperscript{d} Independent Consultant, Bethesda, Maryland, USA

ABSTRACT

Background: Alternative methods for influenza vaccine production are needed to ensure adequate supplies. Methods: Healthy adults 50–64 years were assigned randomly to receive one intramuscular injection of trivalent recombinant hemagglutinin (rHA) or U.S. licensed trivalent inactivated vaccine (TIV) containing H1, H3 and B antigens (Ag) derived from 2007 to 2008 influenza virus strains A/Solomon Islands/03/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004. Each rHA dose contained 45\textsubscript{g} HA/strain of the 2007–2008 FDA-recommended Ag vs. 15\textsubscript{g}/strain for TIV. Antibody (Ab) responses were measured using a hemagglutination-inhibition (HAI) assay at baseline and 28 days post vaccination. Respiratory samples for viral culture were collected from subjects with influenza-like illness (ILI) during the 2007–2008 season in the U.S.

Results: 601 subjects were enrolled. Vaccines were well tolerated. Seroconversion (the percentage of subjects with either (a) a pre-vaccination HAI titer \(\leq 10\) and a post-vaccination HAI titer \(\geq 40\) or (b) a pre-vaccination titer \(\geq 10\) and a minimum four-fold rise in post-vaccination HAI antibody titer) in the TIV and rHA groups, respectively, was obtained in 66\% vs. 72\% for H1; 44\% vs. 61\% for H3; and 41\% vs. 41\% for B. Proportions achieving titers \(\geq 40\) were 96\% vs. 96\% for H1, 75\% vs. 85\% for H3, and 94\% vs. 93\% vs. B. Geometric mean titer ratios at day 28 (TIV/rHA) were 0.77 for H1; 0.58 for H3; and 1.05 for B, respectively. ILI frequencies were low and similar in both groups.

Conclusions: Both vaccines were safe and immunogenic. Ab responses vs. H1 and H3 Ags were significantly higher in the rHA group, with similar responses to B. Furthermore, the FluBlok group had a statistically significantly higher seroconversion rate against influenza A/H3N2 compared to the TIV group.

New Technologies to Meet the Challenge of Pandemic Influenza

Albert Price

ABSTRACT

In the early spring of 2009, a new strain of H1N1 influenza emerged and swept across the globe more rapidly than vaccine producers could keep pace. By the time the pandemic abated in February 2010, the US Centers for Disease Control (CDC) estimated that between 8,500 and 17,600 Americans had died from H1N1 infection, with a disproportionate number of deaths occurring among healthy children and young adults. An estimated 15–25\% of the nation's population was exposed to the virus. However, production of vaccine against this aggressive new influenza strain was agonizingly slow. A total of 18 weeks passed between identification of the new virus and the start of the pandemic's "second wave" — 26 weeks to the peak of that second wave. But the first doses of vaccine did not become available until 26 weeks after strain identification, when spread of the virus was already at its zenith. Vaccine doses sufficient to protect 50\% of the US population became available at 38 weeks, and doses to protect 100\% of the population were available 48 weeks after strain identification.
Comparative immunogenicity of recombinant influenza hemagglutinin (rHA) and trivalent inactivated vaccine (TIV) among persons ≥65 years old

W.A. Keitel\textsuperscript{a,}\textsuperscript{*}, J.J. Treanor\textsuperscript{b}, H.M. El Sahly\textsuperscript{a}, A. Gilbert\textsuperscript{c}, A.L. Meyer\textsuperscript{d}, P.A. Patriarca\textsuperscript{e}, M.M. Cox\textsuperscript{f}

\textsuperscript{a} Baylor College of Medicine, Houston, Texas, USA
\textsuperscript{b} University of Rochester, Rochester, New York, USA
\textsuperscript{c} Ockham Development Group, Inc., Cary, North Carolina, USA
\textsuperscript{d} Children's Hospital Medical Center, Cincinnati, Ohio, USA
\textsuperscript{e} Biologics Consulting Group, Alexandria, Virginia, USA
\textsuperscript{f} Protein Sciences Corporation, Meriden, Connecticut, USA

ABSTRACT
Alternative substrates for influenza vaccine production are needed to ensure adequate supplies. We evaluated the relative safety and immunogenicity of recombinant hemagglutinin (rHA) or trivalent inactivated vaccine (TIV) among 869 ≥65-year-old subjects in a randomized clinical trial. Virologic surveillance for influenza-like illness (ILI) was conducted during the 2006–2007 epidemic. Vaccines were well tolerated. Seroconversion rates vs. influenza A/H1N1 and H3N2 antigens were superior in the rHA group, but were inferior vs. influenza B; however, results for influenza B are confounded since the vaccine antigens were different. ILI frequencies were low and similar in both groups. Studies assessing relative immunogenicity of vaccines using identical B Ags are warranted.

Development of a simple and high-yielding fed-batch process for the production of influenza vaccines

Jamal Meghrous\textsuperscript{a,b}, Wafaa Mahmoud\textsuperscript{a}, Danielle Jacob\textsuperscript{b}, Rick Chubet\textsuperscript{a}, Manon Cox\textsuperscript{a}, Amine A. Kamen\textsuperscript{a,}\textsuperscript{*}

\textsuperscript{a} Protein Sciences Corporation, Meriden, Connecticut, USA
\textsuperscript{b} Biotechnology Research Institute, National Council of Canada, Montreal, Quebec, Canada

ABSTRACT
A robust and reliable GMP-compatible fed-batch process was successfully developed for the production of recombinant hemagglutinin (rHA) proteins by expresSF\textsuperscript{*} cells. The feeding solution, feeding strategy as well as the cell density at infection were optimized to maximize the final rHA production yields without affecting the existing rHA recovery protocol and downstream process. A simple and stable feeding solution was formulated and a rational feeding regimen designed to yield, depending on the rHA baculovirus used, between 2- and 3-fold enhancements in volumetric rHA production with increased specific productivity compared to the batch culture. Recombinant HA from fed-batch cultures could be simply recovered following cell lysis and purified through chromatographic steps. Overall, the increased rHA yield was maintained throughout the whole process. The performance, reproducibility and scalability of the fed-batch process was successfully demonstrated in 12 bioreactor runs of 2- and 10-L working volume using five different rHA encoding baculoviruses.
Design and preclinical development of a recombinant protein and DNA plasmid mixed format vaccine to deliver HIV-derived T lymphocyte epitopes

Leslie E. Walker\textsuperscript{a}, Lo Vang\textsuperscript{a}, Xuefei Shen\textsuperscript{a}*, Penny Post\textsuperscript{b}, Alessandro Sette\textsuperscript{c}, C. Steven Godin\textsuperscript{d}, and Mark J. Newman\textsuperscript{a,}\$  
\textit{a} Pharmexa Inc., San Diego, California, USA  
\textit{b} Protein Sciences Corporation, Meriden, Connecticut, USA  
\textit{c} La Jolla Institute for Allergy and Immunology, San Diego, California, USA  
\textit{d} Bridge Laboratories, Gaithersburg, Maryland, USA

**ABSTRACT**  
Coordinated interactions between helper and cytotoxic T-lymphocytes (HTL and CTL) are needed for optimal effector cell functions and the establishment of immunological memory. We, therefore, designed a mixed format vaccine based on the use of highly conserved HIV-derived T-lymphocyte epitopes wherein the HTL epitopes were delivered as a recombinant protein and the CTL epitopes which were encoded in a DNA vaccine plasmid. Immunogenicity testing in HLA transgenic mice and GLP preclinical safety testing in rabbits and guinea pigs were used to document the utility of this approach and to support Phase 1 trial clinical testing. Both vaccine components were immunogenic and safely co-administered.

Evaluation of the safety, reactogenicity and immunogenicity of FluBlok\textsuperscript{®} trivalent recombinant baculovirus-expressed hemagglutinin influenza vaccine administered intramuscularly to healthy children aged 6 to 59 months

James C. King Jr.\textsuperscript{a,*}, Manon M. Cox\textsuperscript{b}, Keith Reisinger\textsuperscript{c}, James Hedrick\textsuperscript{d}, Irene Graham\textsuperscript{e}, Peter Patriarca\textsuperscript{f}  
\textit{a} Department of Pediatrics, University of Maryland School of Medicine, Baltimore, Maryland, USA  
\textit{b} Protein Sciences Corporation, Meriden, Connecticut, USA  
\textit{c} Primary Physicians Research, Inc., Pittsburgh, Pennsylvania, USA  
\textit{d} Kentucky Pediatric Research Center, Bardstown, Kentucky, USA  
\textit{e} Center for Vaccine Development, St. Louis University, St. Louis, Missouri, USA  
\textit{f} Biologics Consulting Group, Inc., Bethesda, Maryland, USA

**ABSTRACT**  
Background: Recombinant baculovirus-expressed hemagglutinin (rHA [FluBlok\textsuperscript{®}]) influenza vaccine is unique in avoiding production in eggs and its rapid production capability.  
Objective: Compare the safety and immunogenicity of trivalent FluBlok to egg-grown trivalent influenza vaccine (TIV) in children.  
Methods: Healthy children were randomized to receive two doses of study vaccines. TIV (7.5 g HA/antigen), FluBlok-22.5 (22.5 g rHA/antigen), or FluBlok-45 (45 g rHA/antigen) were given to 115 children ages 6-35 months. TIV (15 g HA/antigen) or FluBlok-45 was given to 41 children ages 36-59 months. Safety and reactogenicity data were collected post-vaccination. Serum hemagglutination inhibition antibody (HI) titers were measured before and 28 days after vaccination.  
Results: No serious vaccine-related adverse events occurred and reactogenicity events to equal volumes of TIV or FluBlok were generally similar. However, in the younger children, selected local and systemic symptoms were recorded significantly more frequently to 0.5 mL FluBlok-45 than to 0.25 mL doses of either the FluBlok-22.5 or 7.5 g TIV vaccines. In the younger children, the immunogenicity to TIV was generally significantly superior to FluBlok. Serologic responses to FluBlok were higher in the older children than the
younger group, but were still somewhat lower compared to TIV.

**Conclusion:** These data suggests that FluBlok is as safe but less immunogenic than similar volumes of TIV, particularly in the youngest children. The immunogenicity data is the converse of what has been observed in adults. Further studies examining the immunogenicity of FluBlok in older children are warranted.

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**A recombinant West Nile virus envelope protein vaccine candidate produced in Spodoptera frugiperda expresSF+ cells**


a L2 Diagnostics, LLC, 300 George Street, New Haven, Connecticut, USA
b Protein Sciences Corporation, 1000 Research Parkway, Meriden, Connecticut, USA
c Section of Infectious Diseases, Yale University School of Medicine, New Haven, Connecticut, USA
d Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, Connecticut, USA
e Department of Pathobiology, University of Connecticut, 61 N. Eagleville Road, Storrs, Connecticut, USA

**ABSTRACT**

In this study, a recombinant truncated West Nile virus envelope protein antigen (rWNV-E) was produced in serum-free cultures of the expresSF+ insect cell line via baculovirus infection. This production system was selected based on its use in the production of candidate human and animal vaccine antigens. A defined fermentation and purification process for the rWNV-E antigen was established to control for purity and immunogenicity of each protein batch. The material formulated with aluminum hydroxide was stable for greater than 8 months at 4°C. The recombinant vaccine candidate was evaluated for immunogenicity and protective efficacy in several animal models. In mouse and hamster WNV challenge models, the vaccine candidate induced viral protection that correlated with anti-rWNV-E immunogenicity and WNV neutralizing antibody titers. The rWNV-E vaccine candidate was used to boost horses previously immunized with the Fort Dodge inactivated WNV vaccine and also to induce WNV neutralizing titers in naïve foals that were at least 14-weeks of age. Furthermore, the vaccine candidate was found safe when high doses were injected into rats, with no detectable treatment-related clinical adverse effects. These observations demonstrate that baculovirus-produced rWNV-E can be formulated with aluminum hydroxide to produce a stable and safe vaccine which induces humoral immunity that can protect against WNV infection.

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**Development of a novel recombinant influenza vaccine in insect cells**

Clifton E. McPherson
Protein Sciences Corporation, Meriden, Connecticut, USA

**ABSTRACT**

Influenza is a highly contagious viral respiratory illness that is best prevented through vaccination. Currently, all U.S. licensed influenza vaccines are produced in embryonated chicken eggs. The Baculovirus Expression Vector System (BEVS) technology offers several advantages over existing technology, including an exact match between the circulating virus and the antigen in the vaccine, speed, safety, versatility, and reliable scale-up. The expresSF+ insect cells are grown in the absence of serum and have been extensively qualified for safety according to ICH and U.S. FDA guidance and for suitability for the production of recombinant proteins using BEVS. FluBlok, a recombinant hemagglutinin influenza vaccine, is composed of purified hemagglutinin protein produced using the BEVS technology. FluBlok has been shown to be safe, effective, and efficacious in human clinical studies.
Safety and immunogenicity of a baculovirus-expressed hemagglutinin influenza vaccine: a randomized controlled trial

John J. Treanor, MD\textsuperscript{a}, Gilbert M. Schiff, MD\textsuperscript{b}, Frederick G. Hayden, MD\textsuperscript{c}, Rebecca C. Brady, MD\textsuperscript{b}, C. Mhorag Hay, MD\textsuperscript{a}, Anthony L. Meyer, BS\textsuperscript{b}, Jeanne Holden-Wiltse, MPH\textsuperscript{a}; Hua Liang, PhD\textsuperscript{a}; Adam Gilbert, PhD\textsuperscript{d}, Manon Cox, PhD\textsuperscript{e}

Corresponding Author: John J. Treanor, MD, University of Rochester Medical Center
\textit{a} University of Rochester, Rochester, New York, USA
\textit{b} Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA
\textit{c} University of Virginia, Charlottesville, Virginia, USA
\textit{d} Ockham Development Group, Cary, North Carolina, USA
\textit{e} Protein Sciences Corporation, Meriden, Connecticut, USA

ABSTRACT

Context: A high priority in vaccine research is the development of influenza vaccines that do not use embryo-nated eggs as the substrate for vaccine production.

Objective: To determine the dose-related safety, immunogenicity, and protective efficacy of an experimental trivalent influenza virus hemagglutinin (rHA0) vaccine produced in insect cells using recombinant baculoviruses.

Design, Setting, and Participants: Randomized, double-blind, placebo-controlled clinical trial at three U.S. academic medical centers during the 2004-2005 influenza season among 460 healthy adults without high-risk indications for influenza vaccine.

Interventions: Participants were randomly assigned to receive a single injection of saline placebo (n = 154); 75 μg of an rHA0 vaccine containing 15 μg of hemagglutinin from influenza A/New Caledonia/20/99(H1N1) and influenza B/Jiangsu/10/03 virus and 45 μg of hemagglutinin from influenza A/Wyoming/3/03(H3N2) virus (n = 153); or 135 μg of rHA0 containing 45 μg of hemagglutinin each from all 3 components (n = 153). Serum samples were taken before and 30 days following immunization.

Main Outcome Measures: Primary safety end points were the rates and severity of solicited and unsolicited adverse events. Primary immunogenicity end points were the rates of 4-fold or greater increases in serum hemagglutinin inhibition antibody to each of the three vaccine strains before and 28 days after inoculation. The prespecified primary efficacy end point was culture-documented influenza illness, defined as development of influenza-like illness associated with influenza virus on a nasopharyngeal swab.

Results: Rates of local and systemic adverse effects were low, and the rates of systemic adverse effects were not different in either vaccine group than in the placebo group. Hemagglutinin inhibition antibody responses to the H1 component were seen in 3% of placebo, 51% of 75-μg vaccine, and 67% of 135-μg vaccine recipients, while responses to B were seen in 4% of placebo, 65% of 75-μg vaccine, and 92% of 135-μg vaccine recipients. Responses to the H3 component occurred in 11% of placebo, 81% of 75-μg vaccine, and 77% of 135-μg vaccine recipients. Influenza infections in the study population were due to influenza B and A(H3N2), and influenza A infections were A/California/7/2004–like viruses, an antigenically drifted strain. Seven cases of culture-confirmed CDC-defined influenza-like illness occurred in 153 placebo recipients (4.6%) compared with 2 cases (1.3%) in 150 recipients of 75 μg of vaccine, and 0 cases in recipients of 135 μg of vaccine.

Conclusions: In this study, a trivalent rHA0 vaccine was safe and immunogenic in a healthy adult population. Preliminary evidence of protection against a drifted influenza A(H3N2) virus was obtained, but the sample size was small. Inclusion of a neuraminidase component did not appear to be required for protection.
Dose-Related Safety and Immunogenicity of a Trivalent Baculovirus-Expressed Influenza-Virus Hemagglutinin Vaccine in Elderly Adults

John J. Treanor¹, Gilbert M. Schiff², Robert B. Couch³, Thomas R. Cate³, Rebecca C. Brady², C. Mhorag Hay¹, Mark Wolff⁴, Dewei She⁴, and Manon M. J. Cox⁵

¹ University of Rochester, Rochester, New York, USA
² Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA
³ Baylor College of Medicine, Houston, Texas, USA
⁴ EMMES Corporation, Rockville, Maryland, USA
⁵ Protein Sciences Corporation, Meriden, Connecticut, USA

ABSTRACT

Background: Influenza-virus hemagglutinin (HA) protein expressed in insect cells by recombinant baculovirus is a candidate influenza vaccine.

Methods: In a randomized, double-blind trial conducted in 399 adults > or = 65 years of age, the efficacy of trivalent inactivated influenza vaccine (TIV) licensed for intramuscular injection was compared with that of trivalent baculovirus-expressed HA vaccine administered at doses of 15 microg, 45 microg, or 135 microg of each HA.

Results: Compared with TIV, baculovirus-expressed HA vaccine was safe and induced better serum antibody responses to the H3 component when administered at doses of 45 microg or 135 microg of each HA.

Conclusions: Baculovirus-expressed HA is a safe and immunogenic influenza vaccine in elderly adults.

A recombinant baculovirus-expressed S glycoprotein vaccine elicits high titers of SARS-associated coronavirus (SARS-CoV) neutralizing antibodies in mice

Zhimin Zhou²,*, Penny Post², Rick Chubet², Katherine Holtz², Clifton McPherson², Martin Petric², Manon Cox²

² Protein Sciences Corporation, Meriden, Connecticut, USA
² BC Center for Disease Control, Vancouver, British Columbia, Canada

ABSTRACT

A recombinant SARS-CoV spike (S) glycoprotein vaccine produced in insect cells in a pre-clinical development stage is described. A truncated version of S glycoprotein, containing only the ecto-domain, as well as a His-tagged full-length version were cloned and expressed in a serum-free insect cell line, ExpresSF⁺. The proteins, purified to apparent homogeneity by liquid column chromatography, were formulated without adjuvant at 3, 9, 27, and 50 _g per dose in phosphate saline and used to immunize mice. Both antigens in each formulation elicited a strong immune response after two or three vaccinations with the antigen. Neutralizing antibody titers correlated closely with standard ELISA reactivity against the S glycoprotein. The truncated S protein was also formulated with an adjuvant, aluminum hydroxide, at 1 g per dose (aeadjuvant), and 5 g per dose (aeadjuvant). Significantly enhanced immune responses, manifested by higher titers of serum ELISA and viral neutralizing antibodies, were achieved in adjuvanted groups with fewer doses and lower concentration of S glycoprotein. These findings indicate that the ecto-domain of SARS-CoV S glycoprotein vaccine, with or without adjuvant, is immunogenic and induces high titers of virus neutralizing antibodies to levels similar to those achieved with the full S glycoprotein vaccine.
Development of a Novel Platform TFF System for Insect Cell Culture Harvest

Richard Chubet, James Kacmar, Hank Kopf

ABSTRACT
A single-membrane system was used to clarify insect cells, diafilter the cell concentrates, mix with an elution buffer to release the target protein, and collect the released target protein in the filtrate. The one tank–one module method simplifies and improves the harvest in comparison to multiple centrifugation and filtration steps.

Expression and purification of an influenza hemagglutinin – one step closer to a recombinant protein-based influenza vaccine

Keyang Wang*, Kathleen M. Holtz, Karl Anderson, Richard Chubet, Wafaa Mahmoud, Manon M.J. Cox
Protein Sciences Corporation, Meriden, Connecticut, USA

ABSTRACT
Numerous human infections with avian influenza viruses in Asia in recent years have raised the concern that the next influenza pandemic is imminent. The most effective way to combat influenza is through the vaccination of the public. However, a minimum of 3–6 months is needed to develop an influenza vaccine using the traditional egg-based vaccine approach. The influenza hemagglutinin protein (HA), the active ingredient in the current vaccine, can be expressed in insect cells using the baculovirus expression vector system and purified rapidly.

An influenza vaccine based on such a recombinant antigen allows a more timely response to a potential influenza pandemic. Here, we report an innovative monitoring assay for recombinant HA (rHA) expression and a rapid purification process. Various biochemical analyses indicate that the purified rHA is properly folded and biologically active.

Improved Purification of p55 Protein from Secreted Virus-Like Particles from Baculovirus-Infected Insect Cells by Using an Alternative Selective Precipitation Method

Manon Cox, Rick Chubet
Protein Sciences Corporation, Meriden, Connecticut, USA

ABSTRACT
The Baculovirus Expression Vector System (BEVS) is widely used for the production of a broad variety of heterologous proteins that are often secreted into the culture medium as soluble, biologically active, properly glycosylated, and correctly folded. Downstream purification of a secreted protein is considerably easier due to the absence of many contaminating cellular proteins and nucleic acids in the culture supernatant. The BEVS system has also successfully been used for the production of virus-like particles (VLPs) for a broad variety of proteins derived from many different viruses...
Skewed representation of functionally distinct populations of virus-specific CD4 T cells in HIV-1 infected subjects with progressive disease: changes after antiretroviral therapy

Alexandre Harari, Stéphanie Petitpierre, Florence Vallelian, and Giuseppe Pantaleo
Laboratory of AIDS Immunopathogenesis, Division of Immunology and Allergy, Department of Medicine, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Switzerland

ABSTRACT
HIV-1- and cytomegalovirus (CMV)-specific CD4 T-cell-mediated antiviral immunity was evaluated by assessing the frequency of interleukin 2 (IL-2) - and interferon γ (IFN-γ)-secreting cells following antigen-specific stimulation in blood and lymph node. HIV-1-infected subjects with progressive disease at early stage of infection with no previous history of antiretroviral therapy (ART), subjects with nonprogressive disease, and HIV-negative subjects were studied. On the basis of the ability to secrete IL-2 and IFN-γ, 3 functionally distinct populations of CD4 T cells were identified: (1) IL-2-secreting cells; (2) IL-2/IFN-γ-secreting cells; and (3) IFN-γ-secreting cells. CMV-specific CD4 T cells were almost equally distributed within the 3 functionally distinct cell populations in the 3 study groups as well as HIV-1-specific CD4 T cells in subjects with nonprogressive disease. However, a skewing toward IFN-γ-secreting cells (70% of HIV-1-specific CD4 T cells) was observed in subjects with progressive disease, and IL-2- and IL-2/IFN-γ-secreting cells were almost absent. The frequencies of IL-2- and of IL-2/IFN-γ-secreting HIV-1-specific CD4 T cells were negatively correlated with the levels of viremia. Interestingly, prolonged ART was able to correct the skewed representation of different populations of HIV-1-specific CD4 T cells but was associated with only a partial recovery of IL-2-secreting cells. These results indicate that the composition of the pool of functionally distinct virus-specific CD4 T cells is important for virus control.

Phenotypic heterogeneity of antigen-specific CD4 T cells under different conditions of antigen persistence and antigen load

Alexandre Harari, Florence Vallelian, and Giuseppe Pantaleo
Laboratory of AIDS Immunopathogenesis, Division of Immunology and Allergy, Department of Medicine, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Switzerland

ABSTRACT
The factors responsible for the phenotypic heterogeneity of memory CD4 T cells are unclear. In the present study, we have identified a third population of memory CD4 T cells characterized as CD45RA(+)CCR7(-) that, based on its replication history and the homeostatic proliferative capacity, was at an advanced stage of differentiation. Three different phenotypic patterns of memory CD4 T cell responses were delineated under different conditions of antigen (Ag) persistence and load using CD45RA and CCR7 as markers of memory T cells. Mono-phenotypic CD45RA(-)CCR7(+) or CD45RA(-)CCR7(-) CD4 T cell responses were associated with conditions of Ag clearance (tetanus toxoid-specific CD4 T cell response) or Ag persistence and high load (chronic HIV-1 and primary CMV infections), respectively. Multi-phenotypic CD45RA(-)CCR7(+), CD45RA(-)CCR7(-) and CD45RA(+)(-)CCR7(-) CD4 T cell responses were associated with protracted Ag exposure and low load (chronic CMV, EBV and HSV infections and HIV-1 infection in long-term nonprogressors). The mono-phenotypic CD45RA(-)CCR7(+) response was typical of central memory (T(CM)) IL-2-secreting CD4 T cells, the mono-phenotypic CD45RA(-)CCR7(-) response of effector memory (T(EM)) IFN-gamma-secreting CD4 T cells and the multi-phenotypic response of both IL-2- and IFN-gamma-secreting cells. The present results indicate that the heterogeneity of different Ag-specific CD4 T cell responses is regulated by Ag exposure and Ag load.
Thrombospondin-1 plus irinotecan: a novel antiangiogenic-chemotherapeutic combination that inhibits the growth of advanced human colon tumor xenographs in mice

Giacomo Allegrini, Frederick A. Goulette, James W. Darnowski, Paul Calabresi
Department of Medicine, Division of Clinical Pharmacology, Brown University, Providence, Rhode Island

ABSTRACT
Chemotherapy for the treatment of advanced or metastatic colon cancer, utilizing agents such as 5-fluorouracil (5-FU) and irinotecan (CPT-11), produce a 5-year survival of about 10%. Thus, the identification of new, effective, therapeutic regimens to treat this disease remains critically important. To this end, selected antiangiogenic agents, compounds that inhibit neovascularization, have been shown to produce a modest tumor growth-inhibitory effect with little systemic toxicity. Thus these agents are attractive candidates for use with conventional chemotherapeutic agents to treat this disease. To evaluate this approach, experiments were undertaken to assess the cytotoxic and antineoplastic activity of CPT-11 and the antiangiogenic agent thrombospondin-1 (TSP-1) in the HT-29 model of human colon cancer. These agents were chosen since CPT-11 is a camptothecin analogue efficacious in the treatment of colon cancer and TSP-1 is a human glycoprotein that possess antiangiogenic activity. As expected, in vitro studies revealed that a 5-day exposure to TSP-1 at concentrations up to 130 microg/ml was not cytotoxic alone and did not affect the cytotoxicity of CPT-11, or of its active metabolite SN38, in HT-29 cells. Similarly, in human umbilical vein endothelial cells, TSP-1 alone induced only a slight cell growth-inhibitory effect and did not significantly increase the cytotoxicity of either CPT-11 or SN38. The antineoplastic activities of TSP-1 and CPT-11 were assessed in athymic (nude) female mice bearing advanced subcutaneous xenographs of HT-29 cells. Mice received TSP-1 alone (5-40 mg/kg per day) intraperitoneally (i.p.), CPT-11 alone (100-300 mg/kg, i.p.), TSP-1 (10 mg/kg per day) plus CPT-11 (125 mg/kg), or TSP-1 (20 mg/kg per day) plus CPT-11 (150 mg/kg). TSP-1 was injected daily (Monday through Friday) for 4 weeks (20 injections in total) whereas CPT-11 was administered once weekly on days 0, 7, 14 and 21. By day 28, treatment with TSP-1 alone (5, 10 or 20 mg/kg per day) induced a dose-dependent inhibition of xenograft growth. Further, treatment with 10 or 20 mg/kg per day resulted in an average treated tumor size/control tumor size (T/C) on day 28 of 0.68 (range 0.64-0.71) or 0.58 (range 0.54-0.60), respectively. CPT-11 at all doses significantly inhibited tumor growth with an average T/C value of 0.21 (range 0.15-0.27). However, the 250 and 300 mg/kg regimens induced significant toxicity and mortality. When TSP-1 was combined with CPT-11, a significant (P< or = 0.05) inhibition of tumor growth also was observed (T/C < or = 0.17, range 0.11-0.20). Importantly, this enhanced tumor growth inhibition was obtained without significant toxicity. The therapeutic implications of these findings are discussed.

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA

Pathogenicity and immunogenicity of influenza viruses with genes from the 1918 pandemic virus

Terrence M. Tumpey1, Adolfo García-Sastre2, Jeffery K. Taubenberger3, Peter Palese2, David E. Swayne1, and Christopher F. Basler2

1 Southeast Poultry Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Athens, Georgia, USA
2 Department of Microbiology, Mount Sinai School of Medicine, New York, New York, USA
3 Division of Molecular Pathology, Department of Cellular Pathology and Genetics, Armed Forces Institute of Pathology, Rockville, Maryland, USA
ABSTRACT
The 1918 influenza A H1N1 virus caused the worst pandemic of influenza ever recorded. To better understand the pathogenesis and immunity to the 1918 pandemic virus, we generated recombinant influenza viruses possessing two to five genes of the 1918 influenza virus. Recombinant influenza viruses possessing the hemagglutinin (HA), neuraminidase (NA), matrix (M), nonstructural (NS), and nucleoprotein (NP) genes or any recombinant virus possessing both the HA and NA genes of the 1918 influenza virus were highly lethal for mice. Antigenic analysis by hemagglutination inhibition (HI) tests with ferret and chicken H1N1 antisera demonstrated that the 1918 recombinant viruses antigenically most resembled A/Swine/Iowa/30 (Sw/Iowa/30) virus but differed from H1N1 viruses isolated since 1930. HI and virus neutralizing (VN) antibodies to 1918 recombinant and Sw/Iowa/30 viruses in human sera were present among individuals born before or shortly after the 1918 pandemic. Mice that received an intramuscular immunization of the homologous or Sw/Iowa/30-inactivated vaccine developed HI and VN antibodies to the 1918 recombinant virus and were completely protected against lethal challenge. Mice that received A/PR/8/34, A/Texas/36/91, or A/New Caledonia/20/99 H1N1 vaccines displayed partial protection from lethal challenge. In contrast, control-vaccinated mice were not protected against lethal challenge and displayed high virus titers in respiratory tissues. Partial vaccine protection mediated by baculovirus-expressed recombinant HA vaccines suggest common cross-reactive epitopes on the H1 HA. These data suggest a strategy of vaccination that would be effective against a reemergent 1918 or 1918-like virus.

Hepatitis C Virus and HIV Envelope Proteins Collaboratively Mediate Interleukin-8 Secretion through Activation of p38 MAP Kinase and SHP2 in Hepatocytes

Anuradha Balasubramanian, Ramesh K. Ganju and Jerome E. Groopman
Division of Experimental Medicine, Beth Israel Deaconess Medical Center, Harvard Institutes of Medicine, Boston, Massachusetts, USA

ABSTRACT
Hepatitis C virus (HCV) infects approximately 40% of human immunodeficiency virus (HIV) patients, and the resulting hepatic dysfunction that occurs is the primary cause of death in patients with co-infection. We hypothesized that hepatocytes exposed to HCV and HIV proteins might be susceptible to injury via an “innocent bystander” mechanism. To assess this, we studied the effects of envelope proteins, E2 of HCV and gp120 of HIV, in model HepG2 cells. Upon co-stimulation with HCV-E2 and HIV-gp120, we observed a potent proinflammatory response with the induction of IL-8. Furthermore, our studies revealed that HCV-E2 and HIV-gp120 act collaboratively to trigger a specific set of downstream signaling pathways that include activation of p38 mitogen-activated protein (MAP) kinase and the tyrosine phosphatase, SHP2. Both specific inhibitors of p38 MAP kinase and sodium vanadate, a potent protein-tyrosine phosphatase inhibitor, blocked IL-8 production in a dose-dependent manner. The role of p38 MAP kinase and SHP2 was further defined by transiently overexpressing dominant negative mutants of these proteins into HepG2 cells. These studies revealed that overexpression of an inactive p38 MAP kinase or SHP2 mutant partially abrogated HCV-E2- and HIV-gp120-induced IL-8 production. Further studies revealed that IL-8 induction was not mediated through activation of the NF-kappa B pathway. However, HCV-E2 plus HIV-gp120 was shown to increase the DNA binding activity of AP-1. These results emphasize that expression of the proinflammatory chemokine IL-8, induced by HCV-E2 and HIV-gp120, may be mediated through p38 MAP kinase and SHP2 in an NF-kappa B-independent manner, albeit through AP-1-driven processes.


**Hepatitis C and Human Immunodeficiency Virus Envelope Proteins Cooperatively Induce Hepatocytic Apoptosis via an Innocent Bystander Mechanism**

Neru Munshi, Anuradha Balasubramanian, Margaret Koziel, Ramesh K. Ganju, Jerome E. Groopman
Divisions of Experimental Medicine and Infectious Diseases, Beth Israel Deaconess Medical Center, Harvard Institutes of Medicine, Boston, Massachusetts, USA

**ABSTRACT**

We hypothesized that hepatocytes exposed to hepatitis C virus (HCV) and human immunodeficiency virus (HIV) might be injured via an “innocent bystander” mechanism due to cell-surface binding of viral proteins. To assess this, we studied the effects of HCV envelope protein E2 and T-tropic HIV envelope glycoprotein gp120 on hepatocytes and saw potent apoptosis. Either viral protein alone did not induce this effect. HCV E2 and M-tropic HIV gp120 also induced significant apoptosis. Blocking the CXCR4 receptor led to a reduction in apoptosis. HCV E2 and HIV gp120 acted collaboratively to trigger a specific set of downstream signaling events, including up-regulation of the Fas ligand and dephosphorylation of the anti-apoptotic molecule AKT. These results suggest that hepatic injury may occur in HCV/HIV coinfection through the induction of novel downstream signaling pathways and provide a rationale for therapeutic interventions that interfere with specific receptors and signaling molecules.

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**Presence of Human Immunodeficiency Virus-1-Specific CD4 and CD8 Cellular Immune Responses in Children with Full or Partial Virus Suppression**

The Wistar Institute, Philadelphia, USA

**ABSTRACT**

The present study assessed antiviral T cell immune responses in 48 human immunodeficiency virus (HIV)-infected children with a stable or decreasing CD4(+) T cell counts and different levels of viral control, in the presence or absence of antiretroviral therapy. Children with full (<40 copies/mL) or partial (<50,000 copies/mL) virus suppression and with a history of stable CD4(+) T cell counts had significantly increased levels of anti-HIV CD4(+) T cell lymphoproliferative responses, lower levels of CD38(+), and higher CD8(+)/CD28(+) T cell percentage, compared with those in treated children with a lack of virus suppression (>50,000 copies/mL). Levels of anti-HIV CD8(+) T cell activity, although higher in treated children with a lack of virus suppression, were not significantly different between the groups. Although levels of anti-HIV CD4(+) and CD8(+) T cell responses were not associated, these levels of responses were associated with the percentage of specific T cell subsets. Overall, a history of stable CD4(+) T cell counts, as a result of therapy that imparted full or partial virus suppression, was associated with increased levels of anti-HIV CD4(+) T helper responses and decreased T cell activation.
Analysis of HIV-1 and CMV-specific memory CD4 T-cell responses during primary and chronic infection


Laboratory of AIDS Immunopathogenesis, Divisions of Immunology and Allergic Diseases, Department of Medicine, and Institute of Microbiology, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Switzerland; the Division of Infectious Diseases, San Raffaele Institute, Milan, Italy; and the Laboratory of Virology, University of Geneva, Switzerland

ABSTRACT

CD4 T-cell–specific memory antiviral responses to human immunodeficiency virus type 1 (HIV-1) and cytomegalovirus (CMV) were investigated in 16 patients with documented primary HIV-1 infection (4 of the 16 subjects also had primary CMV infection) and compared with those observed in patients with chronic HIV-1 and CMV coinfection. Virus-specific memory CD4 T cells were characterized on the basis of the expression of the chemokine receptor CCR7. HIV-1– and CMV-specific interferon-γ–secreting CD4 T cells were detected in patients with primary and chronic HIV-1 and CMV coinfection and were mostly contained in the cell population lacking expression of CCR7. The magnitude of the primary CMV-specific CD4 T-cell response was significantly greater than that of chronic CMV infection, whereas there were no differences between primary and chronic HIV-1–specific CD4 T-cell responses. A substantial proportion of CD4+CCR7− T cells were infected with HIV-1. These results advance the characterization of antiviral memory CD4 T-cell response and the delineation of the potential mechanisms that likely prevent the generation of a robust CD4 T-cell immune response during primary infection.

CXCR4/CCR5 Down-modulation and Chemotaxis Are Regulated by the Proteasome Pathway

Aaron Z. Fernandis, Rama P. Cherla, Rebecca D. Chernock, and Ramesh K. Ganju

Division of Experimental Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA

ABSTRACT

Chemokines and their receptors play a critical role in host immune surveillance and are important mediators of human immunodeficiency virus (HIV) pathogenesis and inflammatory response. The chemokine receptors CCR5 and CXCR4, which act as co-receptors along with CD4 for HIV docking and entry, are down-modulated by their respective ligands, MIP-1β/SDF-1α or by the HIV envelope protein, gp120. We have studied the role of the proteasome pathway in the down-regulation of these receptors. Using the yeast and mammalian two-hybrid systems, we observed that the CCR5 receptor is constitutively associated with the ζ subunit of proteasome. Immunoprecipitation studies in CCR5 L1.2 cells revealed that this association was increased with MIP-1β stimulation. The proteasome inhibitors, lactacystin and epoxomicin, attenuated MIP-1β induced CCR5 down-modulation as detected by fluorescence-activated cell sorter analysis and confocal microscopy. The proteasome inhibitors also inhibited the SDF-1α and gp120 protein-induced down-modulation of the CXCR4 receptor in Jurkat cells. However, the inhibitors had no significant effect on the gp120-induced internalization of the CD4 receptor. These inhibitors also blocked cognate ligand-mediated chemotaxis but had no effect on SDF-1α-induced p44/42 MAP kinase or MIP-1β-induced p38 kinase activities, thus indicating differential effects of the inhibitors on signaling mediated by these receptors. These results indicate that the CCR5 and CXCR4 receptor down-modulation mechanism and chemotaxis mediated by these receptors are dependent upon proteasome activity.
Production of a p55gag Particle Vaccine Using the Baculovirus Expression Vector System Technology

Penny L. Post, PhD and Manon M.J. Cox
Protein Sciences Corporation, Meriden, Connecticut, USA

ABSTRACT
Globally, an estimated 36 million people are living with HIV, and some 20 million people have already died of AIDS. Today, there is still no HIV vaccine available. HIV virus-like particles are an attractive vaccine candidate due to their ability to induce both antibody and cytotoxic T-lymphocyte responses. In this article, we describe the development of a production process for an HIV particle vaccine, HIV-1 p55 (gag). The gag precursor protein (p55) is sufficient for assembly and cellular release of retrovirus-like particles. We expressed the p55 gag protein using the BEVS technology in Spodoptera frugipera expersSF+ cells...

Treatment of primary HIV-1 infection with cyclosporin A coupled with highly active antiretroviral therapy

G. Paolo Rizzardi¹, Alexandre Harari¹, Brunella Capiluppi², Giuseppe Tambussi³, Kim Ellefsen¹, Donatella Ciufrèda¹, Patrick Champagne¹, Pierre-Alexandre Bart¹, Jean-Philippe Chave⁴, Adriano Lazzarin³, and Giuseppe Pantaleo¹,²

1 Laboratory of AIDS Immunopathogenesis, Division of Immunology, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Lausanne, Switzerland
2 Division of Infectious Diseases, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Lausanne, Switzerland
3 Division of Infectious Diseases, San Raffaele Scientific Institute, Milan, Italy
4 Clinique de La Source, Lausanne, Switzerland

ABSTRACT
Primary HIV-1 infection causes extensive immune activation, during which CD4+ T cell activation supports massive HIV-1 production. We tested the safety and the immune-modulating effects of combining cyclosporin A (CsA) treatment with highly active antiretroviral therapy (HAART) during primary HIV-1 infection. Nine adults with primary HIV-1 infection were treated with CsA along with HAART. At week 8, all patients discontinued CsA but maintained HAART. Viral replication was suppressed to a comparable extent in the CsA + HAART cohort and in 29 control patients whose primary infection was treated with HAART alone. CsA restored normal CD4+ T cell levels, both in terms of percentage and absolute numbers. The increase in CD4+ T cells was apparent within a week and persisted throughout the study period. CsA was not detrimental to virus-specific CD8+ or CD4+ T cell responses. At week 48, the proportion of IFN-γ–secreting CD4+ and CD4+CCR7– T cells was significantly higher in the CsA + HAART cohort than in the HAART-alone cohort. In conclusion, rapid shutdown of T cell activation in the early phases of primary HIV-1 infection can have long-term beneficial effects and establish a more favorable immunologic set-point. Appropriate, immune-based therapeutic interventions may represent a valuable complement to HAART for treating HIV infection.
Dynamics of viral load rebound and immunological changes after stopping effective antiretroviral therapy

García, Felipe; Plana, Montserrat; Vidal, Carmen; Cruceta, Anna; O'Brien, William A.; Pantaleo, Giuseppe; Pumarola, Tomás; Gallart, Teresa; Miró, José M.; Gatell, José M.

ABSTRACT

Background: This study addresses the dynamic of viral load rebound and immune system changes in a cohort of eight consecutive HIV-1 infected patients in very early stages [all the patients were taking highly active antiretroviral therapy (HAART) and were recruited in the coordinating center from a larger study] who decided to discontinue HAART after 1 year of treatment and effective virologic response. The safety of this procedure and the outcome with reintroduction of the same treatment was also investigated.

Methods: Plasma, cerebrospinal fluid (CSF), and lymphatic tissue viral loads were measured at baseline; lymphocyte immunophenotyping and CD4 lymphocyte proliferative responses to mitogens and specific antigens were assessed. The same antiretroviral therapy was reintroduced as soon as plasma viral load became detectable (above 200 copies/ml).

Results: At day 0, plasma viral load was below 20 copies/ml in all eight patients (and below 5 copies/ml in five of eight patients). A rebound in plasma viral load was detected in all patients from day 3 to day 31 with a mean doubling time of 2.01 (SE 0.29) days. Three out of eight patients achieved a peak plasma viral load at least 0.5 log10 above baseline, pretreatment values. Mutations associated with resistance to reverse transcriptase or protease inhibitors were not detected. After 31 days off therapy, CD4 lymphocytes decreased [mean 45% (SE 4) to 37% (SE 3); P=0.04], CD8+CD28+ lymphocytes decreased [mean 59% (SE 5) to 43% (SE 4); P=0.03], and CD8+CD38+ lymphocytes increased [mean 55% (SE 3) to 66% (SE 4); P=0.009]. Mean stimulation indices of lymphocytes treated with phytohemagglutinin (PHA) and CD3 decreased from day 0 to day 31 from 34% (SE 8) to 17% (SE 9) (P=0.06) and from 24% (SE 8) to 5% (SE 2) (P=0.02), respectively. These changes were mainly contributed by the group of five patients with plasma viral load below 5 copies/ml at day 0. Viral load dropped below 20 copies/ml in all patients after 1 month of restarting the same antiretroviral regimen.

Conclusions: Discontinuation of HAART after 1 year of successful treatment is followed by a rapid rebound of viral load; this rapidly returns to undetectable levels following reintroduction of the same treatment. In patients with more effective control of virus replication (viremia below 5 copies/ml), discontinuation of treatment was associated with more severe impairment of immunologic parameters.

A randomized study comparing triple versus double antiretroviral therapy or no treatment in HIV-1 infected patients in very early stage disease: the Spanish Earth-1 Study

García, Felipe; Romeu, Joan; Grau, Inmaculada; Sambeat, María Antonia; Dalmau, David; Knobel, Hernando; Gomez-Sirvent, Juan Luis; Arrizabalaga, Julio; Cruceta, Anna; Clotet, Bonaventura; Podzamczer, Daniel; Pumarola, Tomás; Gallart, Teresa; O'Brien, William A.; Miró, José M; Gatell, José M.

ABSTRACT

Background: Most current guidelines state that antiretroviral therapy should be considered for HIV-infected patients with plasma HIV RNA > 5000-10 000 copies/ml and CD4 cells > 500 3 106 cells/l. However, there is increasing concern about whether this is the optimal point to begin treatment or whether it is better to delay the initiation to more advanced stages.

Objective: To study the immunological and virological benefits of starting antiretroviral therapy at these early stages.
Methods: A total of 161 HIV-infected asymptomatic patients with CD4 cell count > 500 3 106 cells/l and viral load > 10000 copies/ml were randomly assigned to one of five treatment groups: no treatment, twice daily zidovudine and thrice daily zalcitabine (ZDV-ddC), twice daily zidovudine and didanosine (ZDV-ddI), twice daily stavudine and didanosine (D4T-ddI), or a twice daily three-drug regimen with stavudine and lamivudine and ritonavir. The endpoints were progression to < 350 3 106 cells/l CD4 cells, to < 500 3 106 cells/l with either two Centers for Disease Control class B symptoms or an increase of viral load > 0.5 log10 copies/ml above baseline, or to AIDS or death. In various substudies, the lymphoid tissue and cerebrospinal fluid viral load, development of genotypic resistance, proliferative responses to mitogens and cytomegalovirus, and HIV-1 specific antigens and other immunophenotypic markers were also analysed.

Results: Progression rates to study endpoints within 1 year were greater in the control group (31%) than in all groups receiving antiretroviral therapy pooled together (5%; estimated hazard ratio 7.41; 95% confidence interval 5.72-74.55; P < 0.001). The peak mean viral load decrease was greater in the three-drug group when compared with any of the three groups with a two-drug regimen (2.32, 1.65, 1.72 and 1.84, respectively; P ≤ 0.001). At 1 year, viral load remained below 20 copies/ml in 30 out of 33 patients in the three-drug group (91%) and in only eight out of 94 patients (9%) in two-drug groups (P = 0.001). The peak mean increase in CD4 cells was also greater in the three-drug group than in the double treatment arms (259 versus 85, 144 and 145 3 106 cells/l, respectively; P = 0.001). By comparison, 36% of patients in the three-drug group regimen had to change the therapy as a result of adverse events. Substudies were performed in 60 patients recruited at two sites. Tonsillar tissue HIV RNA was measured in seven patients (two in the two-drug groups and five in the three-drug group) in whom plasma HIV RNA was < 20 copies/ml at 1 year. It was 15 151 and 133 333 copies/mg tissue in the two patients from the two-drug group, < 40 copies/mg tissue in four patients in the three-drug group, and 485 copies/mg in one patient in the three-drug group. At 1 year there was a mean increase of 4.21 ± 2.94% in CD8+CD38+ cells in the control group and a decrease of 9.48 ± 3.36% in the two-drug groups (P = 0.01), and 19.87 ± 3.64 in the three-drug group (P = 0.001 and P = 0.05, for comparisons with control group and two-drug groups, respectively). Although proliferative responses to cytomegalovirus antigens were significantly greater in those receiving antiretroviral therapy, response to HIV-1 p24 antigen was not detected in any patient in either treatment group.

Conclusions: This study supports the recommendation to start antiretroviral therapy with a three-drug combination during very early stages of HIV-1 disease, at least if viral load is above a cut-off point (10000 copies/ml in our study). The risk of progression was sevenfold higher in non-treated patients at 8 months of follow-up. Some immune system parameters improved toward normal values after 1 year of antiretroviral therapy, but the proliferative response of CD4 T lymphocytes against the p24 HIV-1 antigen was not recovered. Therapeutic approaches with more potent, better-tolerated and more convenient regimens will increasingly favour early intervention with antiretroviral therapy.

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HIV-1 specific cytolytic T-lymphocyte activity correlates with lower viral load, higher CD4 count, and CD8+CD38-DR-phenotype: comparison of statistical methods for measurement

García, Felipe; Plana, Montserrat; Vidal, Carmen; Cruceta, Anna; O'Brien, William A.; Pantaleo, Giuseppe; Pumarola, Tomás; Gallart, Teresa; Miró, José M.; Gatell, José M.
Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

ABSTRACT

Objectives: The objective of this study was to use novel statistical methods to determine the correlation between HIV-1-specific cytolytic T-lymphocyte (CTL) activity and HIV-1 plasma viral load, in a blinded study of HIV-infected patients at various stages of clinical disease.

Methods: Peripheral blood mononuclear cells (PBMC) were collected and stored at enrollment and 2 weeks later from 15 HIV-infected individuals who were receiving stable antiretroviral therapy for the previous 6 weeks and during the study period. HIV-1-specific CTL activity was measured using an antigen-specific PBMC in vitro stimulation method. Measurements of plasma viral load, as well as CD4+ and CD8+ T
lymphocytes expressing T-cell activation markers (DR and CD38) were also performed at each time point. CTL activity was quantified using three separate statistical methods: area under the net HIV-specific lysis curve (AUC), lytic units (LU20), and linear regression (LR) of net HIV-specific lysis.

Results: HIV-1 nef-, pol- and gag-specific CTL activity (AUC method) was significantly higher in subjects with a plasma viral load < or = 30,000 RNA copies/ml, than in those with viral load >30,000 RNA copies/ml. When plasma viral load was analyzed as a continuous variable, there was a strong correlation between higher CTL activity and lower viral load for nef (r2 = .77; p < .001), pol (r2 = .63; p < .001) and gag (r2 = 0.75; p < .001) targets by the AUC, but not for the LU20 analysis. Using the LR analysis, which is less dependent on in vitro PBMC growth than the AUC analysis, an independent association was demonstrated between nef- and gag-specific CTL activity and lower viral load. Measurement of CTL activity was also significantly correlated with a higher percentage of circulating CD8+DR-CD38- T lymphocytes.

Conclusions: In this blinded study using an in vitro stimulation of frozen PBMC, higher HIV-1 nef-, pol-, and gag-specific CTL activity correlated with lower plasma viral load, particularly in patients with a CD4 count <500 cells/mm3. Two new statistical methods for estimating CTL activity, AUC and LR analyses, were superior to the standard lytic unit (LU20) method for demonstrating this correlation. These data also demonstrated that higher circulating CD8+ T lymphocytes with a DR-CD38-phenotype, correlate with a lower plasma viral and load and higher HIV-specific CTL activity. This suggests that lymphocytes with this double-negative phenotype may include circulating HIV-specific CD8+ CTL.

Therapeutic immunization with recombinant gp160 in HIV-1 infection: a randomized double-blind placebo-controlled trial: Nordic VAC-04 Study Group

Dr. Eric Sandström MDa, Britta Wahren MDb and Nordic VAC-04 Study Group
a Department of Dermatovenereology, Söder Hospital, S-118 83 Stockholm, Sweden
b Swedish Institute for Infectious Disease Control, Microbiology and Tumourbiology Centre, Karolinska Institutet, Stockholm, Sweden

ABSTRACT

Background: The immune system’s ability to scavenge and destroy detrimental HIV-1 products has an important effect on virion production and the course of infection. In earlier trials of therapeutic immunisation with envelope protein recombinant gp160 (rgp160) we observed a transient positive effect on CD4-lymphocyte counts. This randomised placebo-controlled study investigated whether our preliminary findings represented a potential for a more benign clinical course.

Methods: 835 HIV-seropositive patients from 20 centres in Sweden, Norway, and Finland with CD4-cell counts above 200/microL were randomly assigned to receive 160 microg rgp160 or placebo (alum adjuvant alone) every 3 months for 3 years after an induction period, as well as optimum available treatment. Analyses were by intention to treat.

Findings: 63 of 416 vaccine-group patients and 61 of 419 placebo-group patients reached a primary clinical endpoint (AIDS-defining event or death); the time to first clinical endpoint did not differ between the groups (p=0.864). Significantly fewer vaccine-group patients than placebo-group patients reached the primary immunological endpoint of a decrease of more than 30% from baseline CD4-cell count (157 vs 133, p=0.03). A higher proportion of the vaccine group had CD4-cell counts higher than baseline at 6 months (167 vs 133, p=0.014). HIV-1-specific T-cell immune reactivity was induced in all vaccine recipients studied. No severe adverse events associated with the vaccine were noted during the study. There were significantly fewer deaths among the vaccine recipients than among the placebo-group patients at 2 years, but not at the end of the study.

Interpretation: Therapeutic immunisations with rgp160 have a modest effect on CD4-cell counts, but this treatment alone did not lead to clinical benefit when given in addition to best clinical practice at the time of the trial. Immunisation in conjunction with antiretroviral therapy was also effective, which strongly suggests that a combination with highly active therapy would improve the total effect.
Apoptosis in asymptomatic HIV-1 seropositives immunized with HIV-1 env glycoprotein (gp160): effects of administration of Zidovudine in vivo and interleukin-2 in vitro

University of Rome, La Sapienza, Italy

ABSTRACT
In this paper we report the effects of VaxSyn (Protein Sciences Corp.) immunization on spontaneous apoptosis occurring in vitro after culture of PBMC in medium alone in 30 HIV-seropositive patients enrolled in a double-blind clinical trial that included three groups: treatment with VaxSyn, AZT and VaxSyn, and AZT. Our data show no significant modifications in the levels of apoptosis observed in the three groups over the long-term follow-up (up to 720 days). This was not associated with any significant modifications in other clinical or immunological features. However, analysis of apoptosis performed shortly after the first immunization (at days 3 and 7) showed a significant reduction in the rate of apoptosis in patients receiving AZT and AZT and VaxSyn, as compared with patients receiving VaxSyn alone (30.42 +/- 2.52 SE at day 0 and 23.74 +/- 1.84 at day 3; p = 0.039). Our data also indicate that addition of IL-2 in vitro had a significant inhibitory effect on mortality in all the randomization groups, especially in those receiving AZT (alone or in combination with VaxSyn).
Immune responses elicited by recombinant vaccinia-human immunodeficiency virus (HIV) envelope and HIV envelope protein: analysis of the durability of responses and effect of repeated boosting

M. Juliana McElrath, Lawrence Corey, David Berger, Mark C. Hoffman, Sara Klucking, Joan Dragavon, Eric Peterson and Philip D. Greenberg

Departments of Medicine, Laboratory Medicine, and Immunology, University of Washington School of Medicine, Seattle, Washington, USA

ABSTRACT

Previous studies indicate that immunization with recombinant (r) vaccinia-human immunodeficiency virus type 1 (HIV-1) gp160 and boosting with baculovirus-derived HIV-1 rgp160 results in stronger cellular and antibody responses than those following either vaccine alone. The durability of immunity over 1 year was evaluated in 12 recipients. Both cellular and binding antibody responses remained detectable but diminished, and neutralizing antibodies were absent. To boost immunity, rgp160 was given again 1 year after the initial boost. Reboosting elicited strong HIV-specific lymphoproliferative responses. Binding antibody levels also rose dramatically, and the magnitude of the peak responses was significantly greater following the 2-year than following the 1-year boost. However, neutralizing antibody titers were low (1:10–1:20) and detected in only 4 of 12 persons. Moreover, persistent CD8+ cytolytic responses were not induced. Thus, although repeated rgp 160 boosting after vaccinia-envelope priming can augment selected immune components, an altered regimen may be necessary to achieve protective long-term immunity to HIV-1.

Induction of humoral and cell-mediated anti-human immunodeficiency virus (HIV) responses in HIV sero-negative volunteers by immunization with recombinant gp160


Critical Care Medicine Department, National Institutes of Health, Bethesda, Maryland, USA

ABSTRACT

Development of an effective vaccine for prevention of infection with HIV would provide an important mechanism for controlling the AIDS epidemic. In the current study, the first clinical trial of a candidate HIV-1 vaccine initiated in the United States, the safety and immunogenicity of escalating doses (10-1,280 micrograms) of recombinant gp160 (rgp160), were evaluated in 138 HIV-negative volunteers. Maximal antibody responses, as evaluated by ELISA, were seen after immunization with three doses of 1,280 micrograms rgp160. Responses to some specific epitopes of HIV gp160, including the second conserved domain and the CD4 binding site, were seen more frequently than after natural infection. Neutralizing antibodies to the homologous HIV strain, but not heterologous strains, were induced by this regimen. Blastogenic responses to rgp160 were seen in most volunteers receiving at least two doses of > or = 20 micrograms. These envelope-specific T cell responses were also seen against heterologous strains of HIV. No major adverse reactions were seen after immunization. Thus, rgp160 is a safe and immunogenic candidate HIV vaccine; further studies are needed to determine if it will provide any clinical benefit in preventing HIV infection.
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