

## Plenary Symposia

### PS-1

Mechanisms of Virus Neutralization by Antibodies as Determined Using Structural Approaches. RICHARD J. KUHN. Department of Biological Sciences, Purdue University, West Lafayette, IN 47907. E-mail: kuhn@purdue.edu

Infection with dengue virus results in the production of antibodies, some of which lead to virus neutralization and clearance. In the case of subsequent infection with heterotypic dengue virus, some antibodies can be produced which lead to an enhanced infection (ADE) and a more severe form of the disease. To understand the molecular basis for the activity of neutralizing, non-neutralizing, cross-protective, and enhancing antibodies, we have undertaken a structural approach to investigate the role of this component of the immune system in dengue virus infection. The approach is to identify monoclonal antibodies that have defined biological properties against the virus. Using hybridoma cells, antibody is purified, and Fab fragments are purified and concentrated. These Fab fragments are mixed with virus to form a virus-Fab complex, and cryo-electron microscopy is carried out. The resulting data is then processed into image reconstructions showing the binding of the antibody to the surface of the virus. Combined with an atomic structure of both the E protein and the Fab, a pseudo-atomic structure can be determined. Data will be presented for the binding of several monoclonal antibodies to dengue 2 virus. In addition, several West Nile virus - Fab complexes have been solved. The data that will be presented show that the stoichiometry of binding of Fab to the surface of the virion differs depending upon antibody, and provide insight into the activities of the antibody. Further studies are expected to illuminate the nature of antibody - virus interactions and provide a basis for understanding antibody-dependent enhancement.

### PS-2

Plant-based Vaccines. S. J. STREATFIELD. Fraunhofer USA Inc. Center for Molecular Biotechnology, 9 Innovation Way Suite 200, Newark, DE 19711. E-mail: sstreatfield@fraunhofer-cmb.org

The concept of expressing target antigens in plant tissues to produce plant-based subunit vaccines was proposed about two decades ago and has since received considerable attention, with a large body of academic research, and to a lesser extent more applied industrial research. Most published reports have focused on the expression of target antigens in plant tissues, with the emphasis on expressing sufficient quantities of foreign proteins to evoke immune responses in animals when plant extracts are injected or plant material is orally administered. Mouse studies examining serum, and in some cases mucosal, antibody responses have predominated. In a handful of cases human vaccine candidates have advanced into studies with model species such as ferrets and non-human primates, and into phase I clinical trials. Also, a few animal vaccines have moved into target species trials, and in 2006 the USDA approved a plant-based animal vaccine candidate. A major emphasis remains on attaining much higher levels of expression of target antigens. This allows for higher yields during purification of proteins to be injected, and more practical formulations of proteins to be administered mucosally, including orally. Three general approaches have been followed to express foreign proteins in plants, nuclear transformation, chloroplast transformation and the introduction of viral vectors into plant tissues. In each case several technologies have been applied to raise expression, and for many antigens levels of accumulation are very promising for further vaccine development. With increased expression levels there has been increased emphasis on protein purification and candidate vaccine formulation. Examples of candidate vaccine development will be presented, with the emphasis on viral expression systems.

### PS-3

Plant Cell Culture Production of Recombinant Subunit Vaccines: Biochemical Characterization and Regulatory Approval Process. S. R. WEBB, C. A. Mihaliak, J. Rice, S. E. Keeler, B. W. Schafer, M. R. Badger, A. S. Karnoup, S. A. Young, and M. J. Henry. Dow AgroSciences LLC, Animal Health Research, 9330 Zionsville Road, Indianapolis, IN

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The production of subunit antigens in plants was first described in the public domain in 1990. Since that time it has been well documented in the scientific literature that plants can express vaccine antigens. However, the development and regulatory approval of the first plant-made vaccine took close to 20 years following the pioneering work by Curtiss III and Cardineau (*Oral immunization by transgenic plants* WO 90/02484). In the present study we report on the development and regulatory approval of a plant-cell-produced subunit vaccine to aid in the prevention of Newcastle Disease in poultry. These studies establish that a vaccine produced from a plant cell culture is pure, potent, safe, and efficacious. Detailed characterization of plant-cell produced hemagglutinin-neuraminidase (HN) demonstrates a high level of biochemical, immunological and structural fidelity with purified HN from the native virus. These studies demonstrate that plant cell cultures are a stable, reliable, reproducible and robust production platform for vaccine antigens. The plant-cell-produced system is inherently safe for the production of subunit vaccines. The plant cell system requires no materials of animal origin, has no risk of reversion to virulence or contamination by mycoplasma and has a reduced risk for harboring animal pathogens. These studies have supported the successful completion of the licensure requirements of the USDA Center for Veterinary Biologics (CVB) for the first regulatory approval of a plant-made vaccine.

#### PS-4

Plant Defense Systems Mediated by Protein-carbohydrate Recognition. ELS J. M. VAN DAMME. Department of Molecular Biotechnology, Laboratory of Biochemistry and Glycobiology, Ghent University, Coupure links 653, B-9000 Gent, BELGIUM. Email: elsjm.vandamme@ugent.be

During evolution plants have developed a wide range of sophisticated defense mechanisms to counteract attack by pathogens and herbivory. These include both mechanical and chemical defenses, and allow the plant to survive in the same environment as its attackers. Recent advances in plant disease resistance research revealed some striking parallels between plant defense signalling and the innate immune response in animals and insects. In all cases the perception of a potential pathogen by the host cell initiates a signal transduction cascade that leads to the expression of so called 'immediate early genes'. An overview will be given on the different classes of

proteins expressed as part of the plants' defense system. These include both inducible or constitutive defense mechanisms of higher plants that are relatively conserved during the course of evolution. Accordingly, most plants produce or accumulate structurally and/or functionally similar protective proteins under certain situations, irrespective of their morphological differences. Over the last two decades our research group has focussed on the role of carbohydrate-binding proteins (lectins) in plant defense, in particular against insects. During the last few years important progress has been made in the understanding of the carbohydrate-binding properties of lectins. Since the interaction of a lectin with a complementary carbohydrate is believed to be a prerequisite for any effect of lectins on cells or organisms, our increased knowledge of the carbohydrate-binding properties of plants lectins in turn also leads to a better understanding of e.g. the insecticidal properties of lectins. A detailed discussion on the role of different carbohydrate-binding proteins in plant signalling and defense will be given. In addition, some very recent results on inducible lectins from tobacco involved in plant defense against insects will also be discussed.

#### PS-5

Insect Immunity in *Pseudoplusia includens*: Biowarfare Between Host, Parasitoid and Virus. KEVIN D. CLARK and Michael R. Strand. Department of Entomology, University of Georgia, Athens, GA 30602. Email: kclark@bugs.ent.uga.edu

The insect immune system consists of cellular and humoral components that innately recognize broad classes of foreign intruders. Cellular responses include phagocytosis and encapsulation of foreign invaders by hemocytes, whereas humoral responses refer to molecules like antimicrobial peptides that are released into the hemolymph following systemic infection. Experiments using the moth *Pseudoplusia includens* reveal that capsule formation is initiated by a specific subpopulation of hemocytes called granular cells that recognize a variety of foreign targets. These cells then recruit a second subpopulation of hemocytes called plasmatocytes that are primarily responsible for capsule formation. Recruitment and activation of plasmatocytes depends in part on cytokines like plasmatocytes spreading peptide (PSP), while plasmatocyte adhesion is mediated by adhesion receptors like integrins. In contrast, much less is known about the counter-strategies invertebrate pathogens have evolved to evade or suppress host immune responses. Among the most potent immunosuppressive pathogens of insects are viruses in the family Polydnaviridae which are

associated with parasitoid wasps. In this presentation, we discuss key factors regulating encapsulation and the major virulence factors polydnviruses (PDVs) encode to disrupt this defense response. PDVs such as *Microplitis demolitor* bracovirus (MdBV) disrupt encapsulation by expressing a combination of virulence genes that disrupt cell-cell adhesion and intracellular signaling pathways that regulate specific effector molecules including antimicrobial peptides and phenoloxidases.

#### PS-7

From Cell Lines to Clones to Cows: Development and Production of Recombinant Human Growth Hormone in Transgenic and Cloned Cattle. CARLOS MELO. Bio Sidus, Argentina, Constitución 4234, (1254) Buenos Aires, ARGENTINA. Email: cmelo@biosidus.com.ar

Current production systems for recombinant proteins use bacteria, mammalian cells and, in few cases, yeast. The production costs can be prohibitive for the large-scale use of the derived pharmaceutical in some cases. Other more cost effective systems had to be developed, and transgenic animals are at the moment the best choice. We began to develop primary fibroblasts transfected with the codifying gene for hGH to establish cell lines in the mid term. The cloning process began with those cells, resulting in a first cloned and transgenic female cow, which produced at least 5 g of hHG per liter of milk. As a cow produces 25 L of milk/day during 300 days a year, is possible to obtain 37.5 kg of the recombinant protein per year. Using a purification process having 30% recovery, one cow will produce over 11 kg of pure recombinant protein per year, so only 20 animals could supply 100% of the current worldwide consumption of hGH, with a current value of US\$2 billion per year. The system has several important advantages in development times, costs, production and scale-up. It also has some aspects which should be addressed, such as glycosylation patterns, consistency and viral infections. From the economic point of view, the upstream cost is US\$ for 5-10 g of pure protein, much less than 10% of the current cost with the best systems of cell production available. The current \$25,000 annual treatment cost of a child with pituitary dwarfism will be lowered significantly. Accordingly, children who cannot afford this treatment at current prices will now benefit with this technology. The situation is similar to that with many monoclonal antibodies whose sales prices- due to the fact that they require high doses are thousands of dollars per dose.

#### PS-8

TraitMill™: A High Throughput Functional Genomics Platform for the Phenotypic Analysis of Cereals. CHRISTOPHE REUZEAU, Anabel Sanz, Yves Hatzfeld, Valerie Frankard, Chris De Wilde, Katrien Lievens, Pierre Lejeune, Joris de Wolf, Nico de Wael, Rindert Peerbolte, Wim Van Camp, Marnix Peferoen, and Johan Cardoen. CropDesign NV (A BASF Plant Science Company), Technologiepark 3, 9052 Gent, BELGIUM. Email: christophe.reuzeau@cropdesign.com

The improvement of quality and yield traits in industrial crops is among the most important goals in plant breeding. Yield and quality are quantitative traits, contributed by genetic interaction of multiple alleles. Improvement in these traits have been mostly through conventional breeding. The use of biotechnological tools to modify such quantitative traits is considered as highly challenging. To meet this challenge, CropDesign has developed TraitMill™, a platform that allows high-throughput and high-resolution testing of various natural genotypes, and/or the effect of plant-based transgenes on agronomically valuable traits in crop plants. The focus of the platform is currently on rice, a globally important crop, and a good model for other important cereals such as corn and wheat. TraitMill™ offers a high-throughput prediction of phenotypes from the greenhouse to the field. TraitMill™ is composed of the following key components: a high throughput assembly line platform, comprising allele-design, vector construction, plant transformation, seed increase, and finally population evaluation. The trait evaluation set-up makes use of robots for automated plant transport, digital imaging tools for plant evaluation and proprietary image analysis software for data production and statistical analysis of the results. This phenotype evaluation includes various parameters such as vegetative and root biomass, flowering time, seed yield and seed traits, stress tolerance. A range of interesting product leads has been generated over the last years, including transgenic rice lines showing increased seed yield or increased green biomass. Validation of such phenotypes can be done in several rice genotypes and other cereals. Field-trial experiments showed that the plant phenotype observed in Traitmill™ was often conserved in the field. Thus TraitMill™ can be used as a good proxy for the validation of transgenes, in view for their use for crop improvement. Our results demonstrate the potential of single gene engineering to modify quantitative traits. Moreover, they provide new tools and new alleles for breeders to be integrated in breeding programs and combined with other traits of interest. TraitMill™ is also a tool for validating yield concepts coming from scientific research in universities and public institutes. CropDesign has particular interest to CropDesign are collaborations that aim at identifying and characterizing new ways to increase seed yield and abiotic stress tolerance.