

NORTH AND SOUTH UNITED TO CONQUER VIRAL DIARRHEAS USING INNOVATIVE PASSIVE IMMUNITY STRATEGIES

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Introduction

It is a distinct privilege to be inducted into the Academia Nacional de Agronomía y Veterinaria and to become a member of this esteemed Academy. I am extremely grateful for this honor and to the academy members for nominating me and bestowing this honor on me.

My fortuitous journey to Argentina began 22 years ago in 1987 when I was invited by Dr Alejandro Schudel, then Director of Virology at INTA, Castelar, to visit Argentina to initiate a joint collaboration. The topic was «Rotavirus infections in calves: development and evaluation of maternal vaccines for passive immunity in calves». Passive immunity and enteric viral infections in swine and cattle were two of my major research interests at the Food Animal Health Research Program, Ohio Agricultural Research and Development Center (OARDC), The Ohio State University (OSU) in the USA. At the time, calf diarrhea was a critical problem in both beef and dairy calves, but the major causes were undefined. Our goals were first to identify the dominant pathogens in the field associated with calf diarrhea and deaths and second to develop methods for their prevention and control. To accomplish these goals, we addressed each of the following key questions in collaborative studies conducted in Argentina (INTA) and the USA (OARDC/The Ohio State University).

What is the major calf diarrhea pathogen?

In the dairy and beef industries in the USA, neonatal diarrhea causes economical losses of approximately \$500 million per year. In studies conducted with a visiting scholar, Dr Alejandro Lucchelli from INTA (Castelar, Argentina), we found that group A rotaviruses are the most frequently detected in cases of calf diarrhea and almost 100% of cattle have antibodies to bovine rotavirus (BRV). In Argentina, Biochemist Garaicoechea in our collaborators' INTA lab (Drs Parreno and Fernandez) demonstrated that BRV is associated with 62.5% of calf diarrhea cases. In their studies, she and Dr Costantini determined that BRV was the most frequently detected diarrhea pathogen in beef and dairy herds during a 10-year study period (1994-2003).

What are the dominant rotavirus P (VP4) and G (VP7) serotypes in calves in the USA and Argentina?

Besides diarrhea in young animals, rotaviruses are also the leading cause of severe gastroenteritis in infants and young animals worldwide with about 600,000 deaths in infants per year, mostly in developing countries. Rotaviruses possess a triple-shelled capsid with a dsRNA genome. Like influenza viruses, they have a dual serotype specificity based on the two outer capsid proteins, VP4 (P type) and VP7 (G type), each of which elicit neutralizing antibodies. To develop effective strategies to control rotavirus diarrhea, it is essential to know which are the dominant rotavirus P (VP4) and G (VP7) serotypes in calves essential for inclusion in rotavirus vaccines and if they are the same in the USA and Argentina. Joint studies revealed common rotavirus serotypes circulating in the USA and Argentina. Surprisingly, in both countries, whereas G6P[5] was the prevalent strain in beef herds, a different serotype, G10P[11] was the dominant strain in dairy herds. Thus both of these G and P types are needed for effective BRV vaccines to be used in beef and dairy calves.

How can BRV vaccines be designed to protect young calves from rotavirus diarrhea?

1) ***Live oral vaccines for active immunity.*** Several factors influenced our strategies for development of rotavirus vaccines. Both in Argentina and the USA, calves under 3 weeks of age are most susceptible to rotavirus infection with peak diarrhea and deaths at 6-14 days of age. Thus there is not adequate time for calves to develop active immunity even if vaccines are given at birth. Also because rotavirus infections are widespread, most cows have antibodies to rotavirus in serum and mammary secretions. Interference by pre-existing maternal antibodies causes inconsistent results for active immunization of newborn calves using live oral rotavirus vaccines.

2) ***Maternal vaccines to provide passive immunity to suckling or colostrum-fed calves.***

Immunoglobulin IgG1 is selectively transported from serum into the mammary gland with secretion into colostrum and milk. The IgG1 is selectively transferred via the neonatal Fc receptor (FcRn) into the mammary gland, then secreted into colostrum and milk. Thus parenteral vaccination of pregnant cows represents an effective strategy to increase colostrum and milk IgG1 antibodies. Importantly since most cows have antibodies to rotavirus, maternal vaccines were needed only to boost the pre-existing serum and colostrum antibody titers.

In cattle, no antibodies are transferred across the placenta. Thus, calves are born without circulating antibodies (agammaglobulinemic) which they acquire only via colostrum after suckling. A striking finding of both our studies and those of our Argentine collaborators (Drs Parreno and

Fernandez) was that after transfer of these maternal IgG1 antibodies via suckling to the calf intestine and uptake into blood, these antibodies are then transiently resecreted back into the intestine thereby providing both systemic and local protection.

How can we design rotavirus vaccines to enhance serum IgG1 antibodies in pregnant cows leading to increased colostrum/milk antibodies to rotavirus and passive immunity in calves?

1) Dairy Cattle; colostrum supplemented calves. We initially investigated rotavirus vaccination approaches for pregnant dairy cattle. Observations by Watson and Lascelles in Australia in 1975 revealed new strategies to increase antibody secreting cells in the mammary gland to prevent mastitis in dairy cattle. They proposed that antigen given intramuscularly (IM) with oil adjuvant near involution seeded memory B cells to the mammary gland. Subsequent boosting with antigen via intramammary infusion (IMm) in oil adjuvant during the dry period led to extremely high titers of IgG1 antibodies in serum with their subsequent transport to the mammary gland and secretion into colostrum and milk. We successfully adapted these approaches for live attenuated and inactivated rotavirus vaccines and produced exceptionally high antibody titers in mammary secretions. However a commercial attenuated rotavirus vaccine given IM without adjuvant at a 4 log lower dose than the IM+IMm vaccine failed to significantly increase the antibody titers in mammary secretions above titers in non-vaccinated cows. Our results indicate that the dose, route of vaccination and use of oil adjuvants are critical factors for the success of maternal rotavirus vaccines.

Another important observation was that as little as 1% colostrum supplements from the IM+IMm vaccinated cows provided almost complete passive protection to colostrum-deprived dairy calves against rotavirus challenge (diarrhea and shedding) at 20-25 hrs of age. Colostrum from the IM vaccinated or non-vaccinated control cows did not provide passive protection when fed at similar levels. This protection was correlated with the IgG1 and neutralizing antibody titer to rotavirus in the colostrum.

In collaboration with a visiting scholar, Dr Fernando Fernandez from INTA, we next bioengineered and tested a new generation of rotavirus vaccines. The virus-like particle (VLP) and core-like particle (CLP) recombinant vaccines were produced in a baculovirus expression system by co-expression of multiple rotavirus genes encoding the core and inner capsid (VP2/VP6 CLP) or also the outer capsid neutralizing antigens (VP2/VP4/VP6/VP7 VLP). The rationale for use of such vaccines is that they are safe and noninfectious but they retain the structure and immunogenicity of live rotavirus. Since no harsh inactivating agents are needed like those routinely used to inactivate vaccines, the neutralizing antigens are better preserved. Both VLP and CLP vaccines significantly enhanced IgG1 antibodies in mammary secretions, but only the VLPs with VP4 and VP7 increased the neutralizing antibody titers. Both CLP and VLP vaccines

and an inactivated rotavirus vaccine at least partially passively protected calves against rotavirus diarrhea. However only the VLP vaccine was effective in protecting calves against rotavirus diarrhea and shedding, emphasizing the added contribution of neutralizing antibodies. The finding that the CLP vaccine based on VP6 that induces high titer cross-reactive, but non-neutralizing antibodies provided moderate levels of passive protection suggests that vaccines based on VP6 may also elicit some protection.

2) Beef cattle; naturally suckled calves. In further collaborative studies with Drs Schudel and Fernandez at INTA in Argentina, we initiated a project to develop a maternal rotavirus vaccine for use in beef cows to passively protect their suckling calves. This was a joint effort with Argentine industry (Biogenesis-BAGO), national/international institutions (INTA, OARDC/OSU, Fulbright) and the private sector (field test vaccines at Estancia La Angelica with the collaboration of Ing Agr Romat). A factor that expedited this collaboration was my receiving a short-term Fulbright Fellowship enabling me to conduct a phase of this research in Argentina, including a visit to La Angelica during the field trials. Building on our prior experience with maternal rotavirus vaccines, we designed a chemically-inactivated rotavirus vaccine containing the dominant rotavirus serotypes (G6P[5], G10P[11]) administered in oil adjuvant. This vaccine successfully controlled rotavirus diarrhea in the field trials and was then produced commercially in Argentina. Our dramatic results showed decreased diarrhea and deaths after introduction of the vaccine in Argentina and demonstrated the feasibility and effectiveness of such joint ventures in solving a major disease problem in the field.

Do high titer maternal antibodies in colostrum passively protect neonates but suppress active antibody responses to rotavirus?

It is recognized that high titer maternal antibodies interfere with live oral vaccines. However the mechanisms for this immunosuppression are unclear as are the means to overcome maternal antibody suppression of oral vaccines or induction of active antibody responses. A visiting scholar in my lab, Dr Parreno from INTA initially investigated this question using germfree piglets fed homologous colostrum/milk antibodies from sows immunized with human rotavirus. Advantages to using the germfree piglet model to study immunity to human rotavirus include: 1) They are the only animal model susceptible to human rotavirus diarrhea and the gut lesions resemble those in human infants permitting evaluation of protective immunity to virus challenge. 2) Extraneous enteropathogens (rotaviruses) and maternal antibodies are absent so passive or active immune responses to human rotavirus can be assessed. 3) The piglet gastrointestinal physiology, size, milk diet and mucosal immune responses are similar to human infants.

We found that high titer passive Abs provided partial protection post-primary challenge, but suppressed active IgA gut Ab responses leading to reduced protection post-secondary challenge (Post-Inoculation Day 21). Consequently

multiple doses of live oral vaccines, as formulated for current licensed human rotavirus vaccines, are needed to overcome the immunosuppression. Unfortunately this increases vaccine costs, significantly impacting vaccine use in developing countries where the need is greatest. We also used the germfree piglet model to investigate active intestinal immune responses to human rotavirus vaccines. Dr Ana Sadir, a visiting scholar from INTA with expertise in viral immunology, greatly assisted us in these efforts.

In Argentina, Drs Parreno and colleagues conducted similar studies in calves receiving homologous colostrum from cows vaccinated IM 3X with a live BRV vaccine. Passive colostrum Abs suppressed active Ab responses in calves in a dose-dependent manner. The lowest numbers of Ab secreting cells (all isotypes) were in the IM 3x colostrum fed calves which had the highest serum IgG1 Ab titers. These findings supported and extended the results seen in the germfree piglets. They confirmed that additional approaches (adjuvants, vaccine design, etc) are needed to overcome this problem.

Do heterologous Abs (Llama VHH or chicken IgY) provide passive protection without suppression of active antibody responses of neonatal pigs, infants or calves challenged with rotavirus?

In 2009 a new phase of the collaboration between INTA and OSU was initiated to address the above question. Partial support for this project is provided by a Fogarty International R03 competitive grant from the US National Institutes of Health to Drs Saif, Parreno and Fernandez. Preliminary data generated by Drs Garaicoechea, Parreno and colleagues indicated that the unique single domain recombinant VHH antibodies derived from llamas and generated to bovine rotavirus VP6 neutralized diverse rotavirus serotypes in vitro and passively protected neonatal mice challenged in vivo with murine rotavirus. Thus our current focus is whether heterologous passive antibodies (llama VHH and chicken IgY) will be less suppressive of active antibody responses to rotavirus. Most critically, we will determine if VHH or chicken IgY antibodies against BRV VP6 passively protect germfree piglets against rotavirus diarrhea as a model for infants.

Acknowledgments

I am extremely grateful to the following persons from INTA who contributed their time, intellect and dedicated efforts to the success of these projects. They include the following INTA personnel:

Senior Scientists

Dr. Alejandro Schudel (Former Director, Virology Institute, INTA)

Dr Ana Sadir (Former Director CICV y A, INTA)

Dr. Laura Weber (Principal Investigador, CONICET-INTA)

Visiting Scholars

Dr Fernando Fernandez (Director, Virology Institute, INTA)

Dr. Viviana Parreño (Team Leader, Enteric Viruses Section, Virology Institute INTA)

Dr Maria Barrandeguy (Team Leader, Equine Viruses Section, Virology Institute, INTA)

Masters Student at FAHRP/OSU

Med. Vet. Alejandro Lucchelli

PhD students at FAHRP/OSU

Biochemist Veronica Costantini

Med Vet Sonia Cheetham

Current INTA personnel involved in the Fogarty NIH grant include: Lic Celina Vega

Med Vet Marina Bok, Lic Pablo Chacana, Biochemist Lorena Garaicoechea, Dr. Andres Wigdorovitz and our collaborator, Dr. Jose A. Escribano, from INIA, Spain

Also many thanks to Ing Armando Romat for his cooperation and hospitality during the cattle field trials at La Angelica.

As Marie Curie, the Nobel Prize winner said, «One never notes what has been done; one can only see what remains to be done.» It is gratifying to me that I have been able to pursue my passion for science and to collaborate with and mentor such talented and dedicated students and colleagues from Argentina. I hope that I have enriched their scientific knowledge and lives as much as they have mine. I look forward to continuing to contribute to the human and veterinary scientific communities, in the USA, Argentina and globally.

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